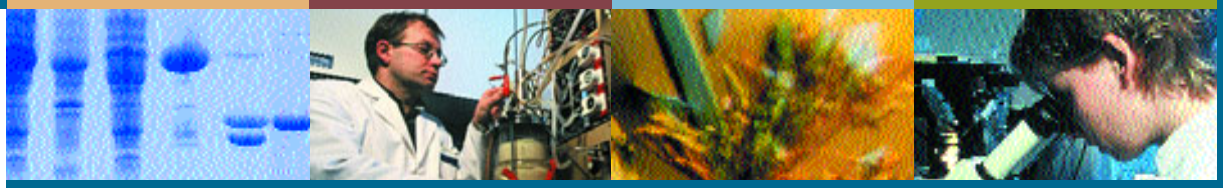
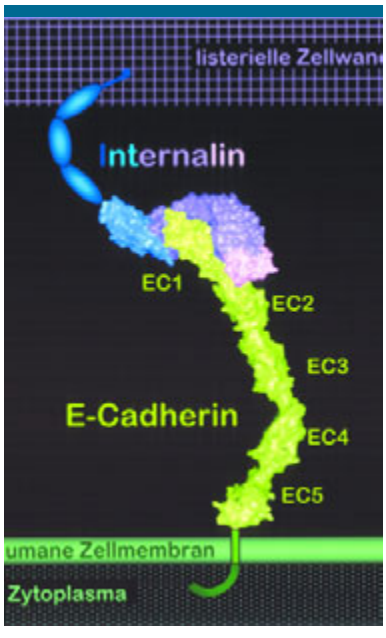


ANNUAL REPORT 2002/2003

GBF

Gesellschaft für
Biotechnologische Forschung
German Research Centre
for Biotechnology



Protein complex between Internalin and E-Cadherin
The bacterium *Listeria monocytogenes* causes listeriosis, an illness that frequently ends fatally in humans. At the surface of the bacterium the protein internalin is expressed in high amounts. In the course of an infection, it interacts specifically with the protein E-cadherin located on the surface of human intestinal cells. Adherance to E-cadherin allows the bacterium to enter intestinal cells to replicate and to subsequently spread throughout the organism. Scientists from the Department of Structural Biology at the GBF have resolved the three-dimesional structure of the complex between internalin and E-cadherin allowing detailed insights to a bacterial infection process at the molecular level.

Photo back cover: Radde

View of the GBF campus from the South. The FORUM, providing venues for seminars, lectures and other functions, can be seen in the left half of the photo. The GBF-building D is located behind the FORUM in the centre of the image. Here, a number of research groups are investigating bacterial pathogenicity and developing vaccines. The building at the right is the new animal facility. In the back, on the left, the upper levels of the German Collection of Micro-Organisms and Cell Cultures (DSMZ) building can be seen.

- *Cover thumbnail pictures from left to right: Determining the structure of proteins by X-ray crystallography requires large quantities of very pure protein preparations. A single protein (blue band, right-hand lane) has been isolated from a crude mixture of cellular proteins (left lane).* | *Large scale protein production is achieved by fermentation of recombinant yeast cells.* | *Crystals of the protein internalin.* | *Protein crystals are observed and selected under the microscope before being prepared for an X-ray analysis.* *Photos (from left to right): GBF, Bierstedt, GBF, Bierstedt*

ERGEBNISBERICHT 2002/2003

GESELLSCHAFT FÜR BIOTECHNOLOGISCHE FORSCHUNG

GBF

Gesellschaft für
Biotechnologische Forschung
German Research Centre
for Biotechnology

FOREWORD

Prof. Dr. Rudi Balling, Scientific Director

Two and a half years have passed since the GBF made the decision to position itself as the "Helmholtz Centre for Infectious Diseases Research" – two and a half years in which we have made great strides and many changes.

The GBF today participates in a large number of national and international research programmes and is a sought-after partner for noted researchers at home and abroad. With the Hannover School of Medicine (MHH), we co-operate in three special areas of research (SFBs). We play a decisive role in both the National Genome Research Network (NGFN) and the EU project "Eumorphia", in which we are responsible for the field "Infections and Allergic Diseases". This new development has been welcomed by the scientific community, business and industry, the media and politics, and has contributed substantially to the success of the "Vakzine Projekt Management GmbH" which, in turn, has generated new impetus into the development of vaccines in Germany.

Such progress is a welcome development, but is no reason to rest on our laurels. What tasks lie ahead for the GBF in the foreseeable future?

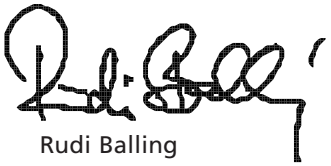
1. The pathogen spectrum that GBF scientists are currently working with needs to be extended. At the moment, discussions are revolving around the analysis of human pathogenic fungi, such as *Aspergillus* and *Candida*, or viral infectious diseases, like hepatitis.

2. Cooperation with universities and clinic partners needs to be expanded. Optimal conditions have been created with the founding of the "Centre for Therapy Research" on the GBF campus. Basic research together with clinical research is jointly conducted with the MHH, Braunschweig Municipal Hospital and the Technical University of Braunschweig.

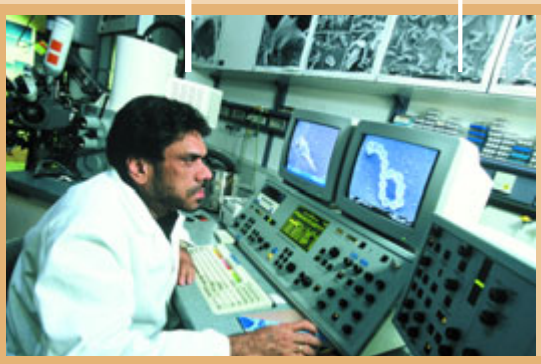
3. Mice as the animal model for human infectious diseases will play a central role at the GBF. New strategies for the diagnosis and therapy of infectious diseases can only be developed using an entire organism. Cell cultures are not sufficient.

4. Future research must be interdisciplinary. The decisive research results in the bio-sciences will come from the intersection of biology, chemistry, medicine, physics and mathematics. We intend to work more on these crossroads and expand, for example, the bio-informatics interface between biology and computer sciences.

We want to approach these core challenges together and clearly focus on them in the years to come. I am certain that in doing so we will be able to move forward quickly, even if financial shortfalls occasionally make that progress a little tougher. I look forward to working with our GBF staff members to achieve those scientific milestones and to celebrate their successes.



Rudi Balling



left: Analysing and studying streptococci | centre: BioS-Laboratory: Alumni applying DNA fragments onto an agarose gel | right: Recently founded: The Centre for Therapy Research in Braunschweig. Prof. Dr. Bernhard Wörmann, medical director of the Medical Clinic with his main fields haematology and cancer at the Municipal Hospital of Braunschweig (le), Prof. Dr. Dieter Jahn, Director of the Institute of Microbiology, Technical University of Braunschweig (ce), and Prof. Dr. Rudi Balling, GBF (ri). *Photographs: Bierstedt (le), Ammerpohl*

The GBF: A Helmholtz Centre for Infection Research

AUTHOR | **Priv.-Doz. Dr. Klaus Schughart | Head of Research and Development and of the Division of Scientific and Technical Services**

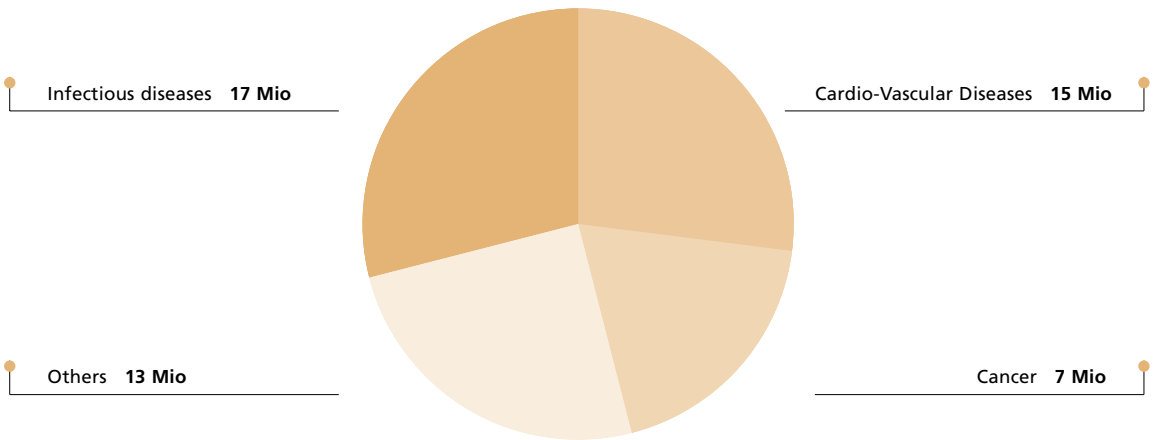
The long-term mission of the GBF is to establish itself as an internationally recognised centre for infection research. In 2002 the GBF set up a new research programme, "Infection and Immunity", within the health research programme of the Helmholtz Association. In this way, the GBF has positioned itself as a centre for infection research in Germany. This development will be continued and extended in the coming years.
At the GBF, the genetic, immunological and environmental factors responsible for the formation and course of infections are investigated. The results of these research activities will provide the basis for the development of new strategies for the prevention, diagnosis and therapy of infectious disease.

Main objectives of the GBF research programme

- Understanding the basic mechanisms of infection and immunity
- Development of new strategies for diagnosis, prevention and therapy of infections

Importance of Infection Research One of the major challenges for health care provision in the 21st century will be to provide more efficient means of fighting infectious diseases. For this, a better scientific understanding of infection and immunity is needed. In the 1960s, bacterial infectious diseases were considered to be defeated. Antibiotics represented a universal and efficient medication to control such infections. But this view has dramatically changed in the meantime. Nowadays, infectious diseases represent a serious health problem, and they are on the rise. Currently, more than seventeen million people die from infectious diseases each year, a third of all deaths worldwide.

Deaths worldwide per year



One of the main causes for the re-emergence of bacterial infections is the development of resistance against antibiotics in microbes. Penicillin, discovered in 1928, was used successfully for many decades. But today, it has almost completely lost its antibacterial effects, because bacterial pathogens developed resistance. In Eastern Europe, multi-resistant strains of the tuberculosis pathogen, *Mycobacterium tuberculosis*, are spreading. Recently, a new variant of the bacterium *Staphylococcus aureus*, resistant against all known antibiotics, was isolated in hospitals in the United States. *S. aureaus* is responsible for life-threatening wound infections after surgery and belongs to the most important hospital pathogens. Therefore, the continuous development of new antibiotics and new classes of antibacterial drugs will be essential for a successful fight against microbes in the future.

In industrialized countries, average life expectancy has increased. But with age, the efficiency of the immune system decreases and, as a result, the susceptibility to and the risk of serious infections rise. Therefore, in the coming years, not only developing countries, but also industrialized countries must expect an increase in morbidity and mortality caused by infectious diseases.

A multitude of current therapies for severe diseases weaken the efficacy of the immune system. This is, for example, the case in cancer patients who have been treated by chemotherapy. Also, after transplantation of organs, the immune reaction must be suppressed with drugs to avoid graft rejection. Under these circumstances, bacterial and viral pathogens have the opportunity to proliferate in a way that is very difficult to control.

Today's increased worldwide mobility results in the spread of pathogens from previously contained reservoirs into regions where these diseases were hitherto unknown. The global dissemination of AIDS, SARS and tuberculosis are some examples.

But pathogens are not only responsible for acute infectious diseases. More and more, correlations between infections and tumour development are being found. For example, infection with the human papillomavirus, HPV, has been recognised as the causative agent for the formation of cervix carcinoma. Infections with the bacterium *Helicobacter pylori* are considered to be the major cause for the development of gastric cancer. Furthermore, associations between infections and immunological diseases are becoming more and more apparent: *e.g.* asthma, rheumatoid arthritis, certain forms of diabetes, multiple sclerosis and allergies. For Streptococci there is clear evidence that an infection with this pathogen can result in the development of an auto-immune reaction resulting in fatal heart disease.

Clinical relevance of infectious diseases

- Increasing resistance of pathogens against antibiotics
- Weakening of immune response with age
- Increased global mobility
- Compromised immune system after cancer therapies and organ transplants
- Correlation between infections and diseases of the immune system or cancer

It is now generally recognised that infectious diseases represent a serious global health problem which can only be met by joint global efforts. Therefore, the WHO, as well as the 6th framework programme of the EU, emphasize the need for significant funding for research and control of infectious diseases. Within the framework of the German National Genome Research Network "NGFN" (Nationales Genomforschungsnetz), the network "Infection and Inflammation" is concentrating on the genetic analysis of infectious diseases. One of the largest private foundations, the Bill & Melinda Gates Foundation, places a special emphasis on the research and development of new vaccines.
Infectious diseases are also an important economical factor. Only recently, the biotech industry started to invest more in the production of anti-infectives and vaccines. The foundation of a Vaccine Management Project Company (Vakzine-Management-Projekt GmbH), initiated by the GBF and supported by the German government, is an important step in accelerating the clinical development of vaccine candidates in Germany.

Epidemic diseases caused thousands of deaths in the past

Source: http://mla-hhss.org/gifs/disease.jpg

Infection Research in the Helmholtz Association
The development and course of an infectious disease represents a complex biological process that is influenced by multiple genetic factors in both the pathogen and the host. In addition, environmental conditions, such as nutrition, medication or life style, play an important role. Therefore, it is necessary to establish multi-disciplinary research projects and to build networks which integrate basic research with pre-clinical and clinical research. Genome research, structural biology, proteome analysis, animal studies and biotechnological production have to be brought together and a sophisticated infrastructure established. Single research institutes can fulfil these requirements only to a limited extend. The Helmholtz Association of research centres, on the other hand, are able to host and integrate many different scientific disciplines and to provide essential infrastructure in one research centre. By focussing a single Helmholtz centre, like the GBF, on infection research, it is possible to provide the critical mass necessary to make substantial progress and to promote the networking of national and international research activities in this field.

Infection Research at the GBF The understanding of infectious diseases and the development of appropriate therapies require a detailed understanding of the pathogen and its interactions with the host at the molecular level. Therefore, basic molecular biology research represents the most essential component of the GBF's research programme. This includes analysis of the interactions between pathogen and host genomes, as well as studies of the environmental factors influencing the development and course of infections. Currently, the GBF concentrates on the analysis of bacterial infectious diseases, and special attention will be given to the development of new antibiotics and vaccines.

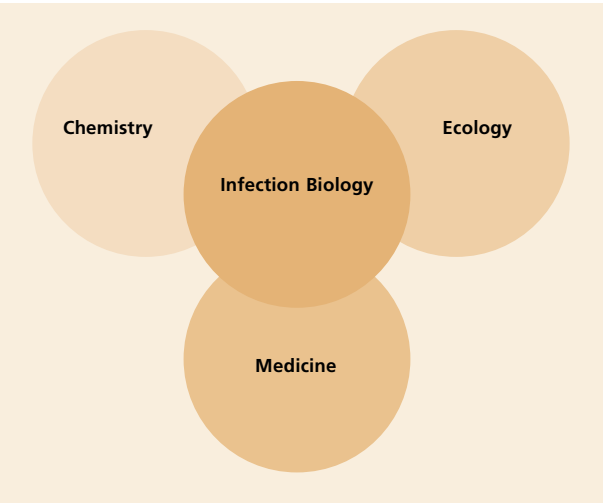
Beyond basic research, the GBF's research activities will contribute to the development of new strategies for the diagnosis and therapy of infectious diseases. Vaccines still represent the most important preventive measure against infections, but in addition, new vaccines are also being developed for therapeutic treatments. Therefore, emphasis will be placed on the identification of vaccine candidates, their pre-clinical validation and the development and optimisation of vaccination strategies.

The GBF participates in the German NGFN research network programme. As one of the core units in the NGFN, the GBF provides technologies and know-how for various partners in the area of infection research. In this context, an "Infection Challenge Platform" has been established at the GBF. This platform performs infection experiments in mice and supplies various mouse strains for the participating network partners. Within "Eumorphia", a multinational European research network, the GBF is developing Standard Operating Procedures (SOPs) for the analysis of mouse mutants in infection assays. These SOPs will serve to improve comparability of results obtained in different laboratories.

Because of its expertise in cellular biology, immunobiology, chemistry, structural biology, genome research, environmental research and the biotechnological production of clinical test material, the GBF is ideally positioned to perform research in the field of infectious diseases.

At the GBF, unique interfaces exist between infection biology and other disciplines, such as chemistry, ecology and medicine. We expect the gain of new knowledge from basic research and its successful translation into medical applications to be particularly promising at these interfaces between disciplines.

Infection and Immunity



A research programme at the interfaces of disciplines

The work of the infection biology groups identifies protein-protein relationships that are essential for host-pathogen interactions. Such interacting protein-protein pairs represent targets for the development of new antimicrobial agents and vaccines. Work in structural biology at the GBF describes molecular interactions at the atomic level. This knowledge serves as the starting point for the design of small molecules or neutralizing antibodies, which can inhibit essential host-pathogen interactions and thereby fight or prevent infection. Chemistry groups at the GBF provide combinatorial chemical libraries and new natural products, which are screened for biologically active compounds that can selectively inhibit protein-protein interactions during the infection process.
In most cases, the course of a bacterial infection is not determined by a single pathogen, but involves bacterial communities. Therefore, infections have to be considered as ecological processes. In the past, the GBF has accumulated a lot of expertise in the analysis of microbial communities in the environment. This knowledge will now be applied to study clinically relevant bacterial biofilms. For example, patients that have been surgically treated for liver cancer may require a stent, which allows draining of the bile into the intestine. Over time, these stents become colonized by bacterial communities, which form biofilms and which eventually plug the lumen, so that replacement surgery is necessary. One goal of our research efforts is to understand the principle mechanisms underlying the formation and maintenance of such biofilms. The knowledge gained can be used subsequently to develop new intervention strategies to avoid the initial formation of bacterial biofilms. In the more distant future, the influence of nutrients on microbial communities in the intestines and its colonization by pathogens will also be investigated at the GBF.

To speed the transfer of promising drug candidates from the pre-clinical research stage to clinical studies, it is necessary for scientists in basic research to work closely with clinical research groups. The establishment of a Centre for Therapy Research on the GBF campus, in cooperation with the Technical University of Braunschweig, the Medical University of Hannover and the Municipal Hospital of Braunschweig represents an important milestone in this direction. Here, clinical research groups and basic research groups will closely work together.

Analysing DNA-chips: results generated by our technological platform

Photo: Bierstedt

Technological platforms at the GBF

- Animal facility (SPF breeding, knockout technology, infection unit)
- Analytic instruments (nuclear magnetic resonance analysis, X-ray diffraction analysis, mass spectrometry, electron microscopy)
- Expression arrays
- Peptide synthesis and sequencing

The GBF provides a multitude of technological platforms that support internal research projects and also cooperation projects with external partners. More details can be found in other sections of this annual report.

Research Programme "Infection and Immunity"

The main emphasis of the research programme of the GBF is on "Infection and Immunity". In this research programme we try to understand the principle biological mechanisms underlying the development of an infectious disease. This involves basic research on model organisms, as well as on clinically relevant pathogens. The causes of pathogenicity in an infectious agent and the defence mechanisms of the host, in particular the generation of immunity, will be investigated. These studies will lead to a better understanding of the molecular and cellular processes underlying the development of an infectious disease. They will allow us to better understand why only certain microorganisms cause disease, while other, closely related variants, do not.

The results obtained will then form the basis for the development of new strategies to diagnose, treat and prevent infectious diseases. In addition, the knowledge gained from studies of the immune system will be used to design new strategies for the treatment of infections, and also for other human diseases, such as autoimmune disease and cancer.

The GBF's research activities will mainly be performed within the framework of the Helmholtz research programme "Infection and Immunity". This research programme has been established by the GBF in collaboration with the Research Centre for Environment and Health (GSF) in Munich. The research activities of these two centres will complement each other, with the GBF concentrating on bacterial infectious diseases and the GSF focusing on viral pathogens.

Infection and Immunity

- Microorganisms
- Pathogenesis
- Immunobiology
- Prevention and Therapy
- Biotech Facilities

• *More details on individual research projects can be found in other sections of this annual report.*

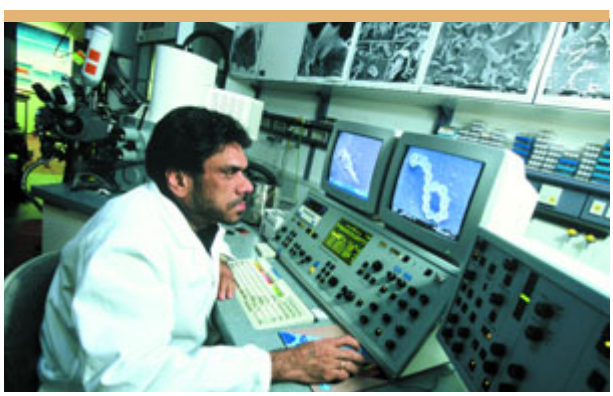
• *Research with model organisms: the nematode* C. elegans

Photo: Bierstedt

The programme "Infection and Immunity" includes the research topics "Microorganisms", "Pathogenesis", "Immunobiology", and "Prevention and Therapy". In addition, the GBF will establish a national and international research platform (Biotech Facilities) providing biotechnological products for clinical and basic research.

Microorganisms The goal of this research topic is to identify and characterise virulence factors in microbial pathogens, and to understand the mechanisms of the formation of antibiotic resistance. Furthermore, the structure of virulence factors and their interaction with host proteins is being investigated at the atomic level.

Streptococcus bacteria are an example from the GBF research programme. It is estimated that 40 million children between 5 and 15 years are infected with these bacteria every year. The bacteria cause diseases such as scarlet fever and tonsillitis. Without proper treatment, life-threatening damage may result. As a consequence of infection with Streptococcus bacteria, about 15 million children suffer from rheumatic heart disease, from which approximately half a million die every year. Apart from elucidating the infection mechanisms, the GBF research aims at the development of a protective vaccine.



• *Analysing and studying streptococci*

Photo: Bierstedt

Pathogenesis A more detailed understanding of the course of an infection process will form the basis for designing new therapeutic strategies. The multiple interactions between pathogen and host are thus being investigated at the cellular level. Bacterial infections, which enter the host via the mucosal surfaces of the digestive or respiratory tract, are of particular interest.

Immunobiology In the research area "Immunobiology", the principle cellular and molecular mechanisms of immune response are investigated. The goal is to understand how an effective immune response can be induced and maintained, *e.g.* to discover which decision processes are critical in triggering either an immune response or the formation of tolerance. Of particular interest is the question of how certain pathogens manage to bypass the immune system.

Prevention and Therapy Work in this research area is directed towards the development of new strategies for treating and preventing human diseases. For this purpose, natural products and chemical libraries are screened for new anti-infective molecules. Antigen delivery systems and adjuvants for vaccines are developed, and new therapies, which are based the modulation of the immune system, are investigated. Vaccine candidates are being developed and tested in clinical studies in collaboration with clinical research groups.

Mice play an important role for immunobiological research projects

Photo: Bierstedt

Biotech Facilities GBF Biotech Facilities serves as a national and international technology platform. It provides biotechnological production processes for industry and public research institutes. In addition, biological products for clinical studies are produced according to GMP (Good Manufacturing Process) and pharmacological standards. Furthermore, biological research material, which is not available commercially, is prepared on a large scale for basic research.

GMP is a prerequiste for the production of vaccines

Photo: Bierstedt

Klaus Schughart born 1956, studied biology at the University of Köln, 1986 PhD at the Institute of Genetics, University of Köln, 1987 – 1989 Postdoctoral Fellow in the Laboratory of F. H. Ruddle, Yale University, New Haven, USA, 1990 – 1994 Junior research group leader at the MPI of Immunobiology, Freiburg, 1994 Habilitation in Genetics, University of Freiburg, 1995 – 1996 Research group leader at the GSF, Munich, 1995 "*Venia Legendi*" for genetics at the University of Freiburg, 1997 – 2001 Head of the Department of Molecular and Cellular Biology, Transgene S.A., Strasbourg, France, since 2002 Head of Research and Development and the Division of Scientific and Technical Services, GBF

ACKNOWLEDGEMENT *I would like to thank all colleagues who participated in the preparation of the PoF application "Infection and Immunity", the strategic paper 2002, and the R&D programme 2003. These contributions have formed the basis for this article. In particular, I wish to thank Thomas Gazlig, Prof. Dr. Rainer Jonas and Dr. Christopher Schippers for valuable suggestions and comments.*

Highlights 2002

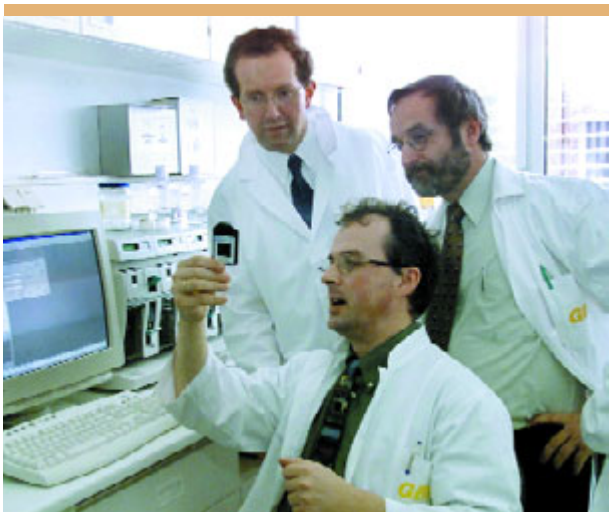
AUTHOR | **Dipl.-Journ. Dipl.-Biol. Thomas Gazlig | Head of Public Relations**

Germ Research in Lungs and Intestines Two new SFB (special research areas) designed to study the interaction between microbial pathogens and human mucous membranes will be funded by the German Research Foundation (DFG). SFB 1922 – "The Pathobiology of Intestinal Mucosa" – will examine abnormal processes on the intestinal mucous membrane as well as the effects of probiotica – bacteria that support and improve the health and immunity of the intestinal surfaces. SFB 1921 – "Immune Reactions in the Lungs Caused by Infections and Allergies" – will study how bacteria, viruses and fungi attach to bronchial mucosa and how antibodies are controlled in the respiratory tract. Besides the GBF, the Hanover School of Medicine (MHH), the Hanover School of Veterinary Medicine (TiHo), the University of Hanover and the Fraunhofer Gesellschaft will participate in these research projects.

The German Government's Vaccine Initiative The Federal Ministry of Education and Research (BMBF) has announced plans to accelerate the development of new vaccines in Germany – and has placed this "Vaccine Initiative" in the hands of the GBF. Research Minister, Edelgard Bulmahn, personally unveiled the plans for the project in Hanover in December 2002. The BMBF has earmarked 25 million Euro for the next five years to accelerate the development of promising vaccines and to promote Germany as a leader in vaccine research and development. The principle partners in pursuit of this goal are the non-profit German Vaccine Research Foundation and the for-profit "Vakzine Projekt Management GmbH", which is operated under the auspices of the foundation and the development fund of the GBF.

Vakzine Projekt Management GmbH

"Vakzine Projekt Management GmbH"



Recently founded: The Centre for Therapy Research in Braunschweig. Prof. Dr. Bernhard Wörmann, medical director of the Medical Clinic at the Municipal Hospital of Braunschweig (le), Prof. Dr. Dieter Jahn, Director of the Institute of Microbiology, Technical University of Braunschweig (ce), and Prof. Dr. Rudi Balling, GBF.

Photo: Ammerpohl

Research Know-How for Clinics and Hospitals The development of new concepts for the diagnosis and therapy of diseases: this is the goal of the Centre for Therapy Research, which the GBF jointly founded with the Municipal Hospital of Braunschweig and the Technical University at Braunschweig. Additional expertise is provided by another partner in this endeavor: the Hanover School of Medicine (MHH). Accelerating the transfer of empirical findings generated by basic research to the clinical development is one of the key goals of the Centre for Therapy Research. The focus of research will be studies to promote a better understanding of infections in immune-suppressed patients and the development of therapies to treat infections.

Mouse Genetics for Health Research In the past year, the GBF became a partner in the new European research programme "EUMORPHIA". The European Union is providing 12.3 million Euro for the programme over the next three years, of which 730,000 Euro has been earmarked for Braunschweig. Research laboratories from eight countries are participating in the programme. The project will catalog genetically altered mice that are relevant for health research. The GBF will focus on questions concerning the biological aspects of infections. Mice will be bred and studied that are especially susceptible to bacterial infection or that demonstrate auto-immune or allergic reactions. The GBF will be working closely with the National Research Centre for Environment and Health (GSF) in Munich, the second German institute involved in the EUMORPHIA programme.

Serving mankind: The mouse is an important model organism for researchers at the GBF to study infectious diseases. Only in living organisms complex diseases can be investigated successfully.

Photo: Bierstedt

Worldwide Searching for Pharmaceuticals in the Seas Extracting biologically active compounds from marine organisms was the key aim of the research project "Marine Biotechnology" in Lower Saxony. Nineteen work groups from different universities and research institutes participated in this joint project, funded by the Volkswagen Foundation. The project ran over a five year period and ended in 2002. A team headed by Dr. Irene Wagner-Döbler from the GBF, isolated many new bacteria which were catalogued using molecular biological methods. The GBF researchers gave special attention to proteobacteria, toxin builders in the *Roseobacter*-group, which are notorious for causing algal blooms. A series of promising ingredient agents was found by Dr. Wagner-Döbler and her team in these marine bacteria. Some with interesting pharmaceutical qualities, will now be tested more thoroughly.

Navigatin through the Sequencing Jungle The gene search engine "NGFN-BLAST" now provides scientists with an easier way to find the relevant information in previously identified genetic sequences. It is the first publicly accessible service of its kind in Germany and allows sequence comparisons between the genes of different mammals, *e.g.* between humans, mice and rats. The BLAST (Basic Local Alignment Search Tool) server is part of the GBF's research project in the National Genome Research Network (NGFN). Members of NGFN receive preferred access to data and can build user-specific sub-groups in the data bank, thereby considerably accelerating searches and supplying users with only the relevant data they need.

Physicians Seminar: Genetics in Emergency Medical Care The GBF and MHH organized a crash course in molecular medicine and genome research for young doctors. The seminar was designed as supplemental medical training. Currently, only very few doctors have any practical knowledge of genome research methods. This course focused on possible and practical applications for daily hospital routines. The participants learned how to use gene chips or PCR for a rapid diagnosis in the emergency room. For example, one exercise was how to detect the presence of life-threatening diarrhea in children who had been in contact with pathogenic *E. coli* bacteria.

Training Programme: "From Genes to Vaccines"
The goal of the International Training Programme (ITP) at the GBF was to provide scientists from developing nations with the expertise to advance infection and vaccine research in their home countries. The 12 post-doctorate participants, who were trained in Braunschweig from August 5th through September 13th, 2002, are now working in their own countries to promote infection and vaccine research and communicate the special knowledge gained here in Germany. The ITP was able to provide them with the tools needed to set up similar training programmes at home.

International experiments in the laboratory: The participants of the ITP-Course work about the development of vaccines

Photo: Ammerpohl

Biotech Course for Southeast Asian Scientists In 2002 the GBF organized its fourth training programme for young scientists from Southeast Asia, together with the Carl-Duisberg Society (CDG; now called InWEnt), BioRegioN and the Central Placement Office (ZAV). Twenty researchers from universities and enterprises from four ASEAN countries, Thailand, Indonesia, the Philippines and Vietnam, participated in the "Industrial Biotechnology" programme. At the GBF, the young scientists attended a special introductory course in modern biotechnology from May 27th through June 28th. The course was preceded by a two-and-a-half months crash course in German language and culture. Afterwards, the young scientists spent several months at companies and research labs in the region to expand on the expertise gained from the biotech course.

Conference on Infections for Biomedical Researchers
Infections as a cause for chronic illness and disease was the focus of a GBF symposium in October 22–23 2002. The international conference was sponsored by the Clinical Biomedical Research Alliance (KBF) which welcomed numerous leading scientists and researchers involved in fundamental pre-clinical and clinical research in this field. Among other subjects, the symposium discussed the contribution of infections to cancer, chronic liver diseases and chronic inflammation, as well as cardiopulmonary and neurological diseases.

The World Congress of Streptococci Researchers
For the first time, the GBF, together with the All India Institute of Medical Science, hosted the International Lancefield Symposium. Every three years streptococci researchers from around the world come together for this conference to discuss their research results. The symposium was held in Goa, India, in October 2002. For the first time ever the conference took place in the developing world. This time about 300 participants focused their attention on the theme: "The Fight against Rheumatic Fever and Rheumatic Heart Disease." Despite the availability of antibiotic treatments for streptococci, these bacteria remain a serious health problem and exert an enormous social and economic burden in many developing countries. Particularly dangerous are secondary infection, such as rheumatic heart disease. Alone in India, about six million children suffer from this disease.

Beneath palms: The announcement of the Lancefield Symposium in Goa, India

Photo: Gazlig

GBF Coordinates Crystallography Meeting The annual "Heart of Europe BioCrystallography Meeting" this time around was organised by the GBF. Dr. Dirk Heinz, head of the Department of Structural Biology, was in charge. The meeting in Goslar attracted researchers from Germany, Poland and the Czech Republic. The BioCrystallography Meeting provides doctoral students and young researchers a platform for presenting their work. Among other things, the team around Dirk Heinz and his team presented their results on the structural analysis of internalin, a protein used by *Listeria* bacteria to recognize the surfaces of human intestinal mucous membrane cells.

A Trade Fair for Lab Equipment For the first time visitors could inform themselves at a trade fair at the GBF-Forum. The laboratory equipment company, Omnilab, which leased space in the GBF-Forum, organized a specialist exhibition for biotechnology devices and products. At some 60 stands, domestic and foreign suppliers exhibited everything from microfilters, to chromatographs and laboratory furniture. The Lab Fair attracted about 500 visitors, from institute directors to laboratory technicians.

The trade fair, organized by Omnilab, attraced many visitors

Photo: Omnilab

A Nationwide Genome Telephone Under the motto "The Genome and Behind", project leaders of the German Human Genome Project (DHGP) discussed current developments in genome research in the GBF Forum. A highlight of the event was the so-called genome telephone. For two days, leading German genome researchers were available at a toll-free number to answer questions posed by the general public. The genome telephone was organized by the DHGP, the GBF, the Human Research Development Association and the Braunschweig branch of Deutsche Telekom.

At the GBF-genome telephone: Prof. Dr. Jens Reich.

Photo: Gazlig

Out of the Drawer and onto the Market Frequently, patented inventions are not always efficiently marketed and end up costing more than they earn. To improve the return from patents, Ascenion GmbH has been marketing the GBF's patent portfolio since May, 2002. Four Helmholtz centres (GBF, MDC, GSF and DKFZ) have joined forces to found a Life Science Foundation. The Ascenion GmbH is solely owned and operated by the foundation that runs its headquarter in Munich. Branch offices are located in Braunschweig and Berlin. Revenues are returned from the foundation back to the participating Helmholtz centres.

The Centre for Biotech Start-Ups The idea for the Centre for Biotech Start-ups was initiated by the City of Braunschweig and built in just one year. The investment costs were shouldered by the city and the state of Lower Saxony. The laboratory and office building, located close to the GBF campus, has a total area of 4,000 square meters (43,000 sq. ft.), of which 1,400 sq. meters is laboratory space and 2,600 sq. meters is reserved for start-up company offices.

Front view of the BioTec Business Incubator Facility

Photo: Stadt Braunschweig

Grants for Biotech Companies The BioProfile Functional Genome Analysis, an initiative of biotech companies and research institutes of Lower Saxony, has launched its funding programme. The initiative's goal is to promote the practical application of human genome research in infection biology, stem cell biology and neurobiology. Funds from the Federal Ministry of Education and Research (BMBF) have been made available. The initiative's jury includes experts from across Germany as well as the director of the GBF, Prof. Dr. Rudi Balling. The first two projects recommended to the BMBF for funding by the jury and the initiative's board are an early diagnosis system for diabetes (Mosaiques diagnostics, Hanover) and a knockout method for mice genes (DeveloGen, Göttingen). Both biotech firms will receive grant money totaling about one million Euro.

Biotech Days at the GBF FORUM The fourth Biotechnology Days, sponsored by the BMBF, took place at the GBF on May 13 – 14, 2002. More than 400 visitors – mostly decision-makers from business and industry, finance, politics, government agencies and the scientific community – were gathering at the GBF FORUM. The business consultant firm, Ernst & Young, also presented its German Biotech Report 2002.

Prize-Winning Scientists Several GBF researchers received awards and citations for their work in 2002. Dr. Hansjörg Hauser received the Boltzmann Award for Cytokine Research. Dr. Rolf Müller was the winner of the Dechema Young Scientist Prize for Natural Science. The Poster Prize of the second international symposium "Antioxidants in Nutrition & Therapy" from the Society for Free Radical Research held in Indonesia, went to Heike Budde.

Inhoffen Medal for Peptide Design Professor Dr. Horst Kessler, TU Munich, received the Inhoffen Medal for his work on peptide design using NMR spectroscopy. The award, donated by the Technical University of Braunschweig and the GBF, honoured in particular the multidisciplinary approach of his work. The research fields of Prof. Kessler are molecular design, the synthesis and structure of peptides and peptide-like substances.

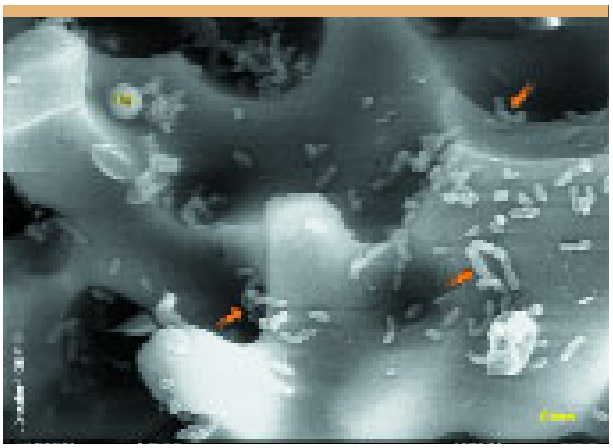
Fourth BMBF-Biotech Days at the GBF: Prof. Dr. Rudi Balling, Edelgard Bulmahn, Federal Minister for Education and Research, and Sigmar Gabriel (right), former Prime Minister of the State of Lower Saxony, during the Opening Ceremony.

Photo: Gramann

Awards to Young Scientists The Development Fund of the GBF honoured excellent PhD-thesis works: Nicole Glaser studied naturally occurring variations of the anti-cancer molecule epothilone that are produced by different bacteria species. Edelweiß Markworth and Andreas Timan investigated the structure and effect of ATP-sensitive ion channels in the cell wall. These channels coordinate the intra-cellular signalling of external stimuli and are involved in muscle contractions or secretion of macro-molecules. Diseases like Diabetes type II are associated with defects in ion channels.

The Fritz-Wagner Prize 2002 was awarded to Dr.-Ing. Christina Mundhenke from the Institute of Mechanical Engineering of the TU Braunschweig. She investigated in which way the cutting of organic materials, like apples or avocados, into smaller pieces influences their degradation in sewage plants.

Mercury Remediation: New Process Ready for the Market Three GBF scientists won the Erwin Schroedinger Prize for interdisciplinary research at the end of 2001: Dr. Irene Wagner-Döbler, Prof. Dr. Wolf-Dieter Deckwer and Prof. Dr. Kenneth Timmis. The scientists developed their idea to purify mercury-contaminated industrial waste water with bacteria into a marketable product. To refine their procedure they worked together with ecologists, bio-technicians and microbiologists. The Donor's Association for German Science honors such interdisciplinary research efforts every year with the 50,000 Euro Erwin Schroedinger Prize.



Microbial remediation: The electron-microscopic photograph shows the rod-shaped bacteria Pseudomonas putida *attached on Siran, a ceramic support material*

Photo: GBF

GBF Staff Helps Flood Victims GBF staff members rolled up their sleeves to help flood victims in Eastern Germany. Some fifty GBF employees went to Magdeburg and helped to fill sandbags and to reinforce dikes. A cheque of 10,000 Euro was presented to the Braunschweig Deaconess Relief Agency to support social aid and relief efforts in Raguhn in neighboring Saxony-Anhalt. GBF expertise was also in demand because the flood waters had left behind a good deal of sludge and bacteria. Microbiologists brought their mobile environment lab to the flooded area around Hitzacker. Dr. Wolf-Rainer Abraham and Dr. Dirk Wenderoth examined sludge and water samples for disease-causing bacteria, such as *Salmonella* and pathogenic *E. coli* strains.

Dedication: Many helpers carry sandbags as a protection against flooding near Magdeburg

Photo: Gazlig

Biotech Laboratory for Schools In an effort to generate more interest among young students in science and research, the GBF, together with Braunschweig Technical University and the Braunschweig district government, has set up a school laboratory for biotechnology, known as BioS. The project is also supported by the Lower Saxony state government and the Helmholtz Association. The LB Public Foundation is a co-sponsor. The biotechnology school laboratory has been open to senior high school students since spring, 2002. The BioS lab offers students an opportunity to gain basic biotechnology skills by conducting experiments that cannot be done in ordinary school facilities. BioS is run by two high school teachers who oversee experimental courses for groups of up to 24 pupils.

BioS-Laboratory; Alumni are applying DNA fragments onto an agarose gel.

Photo: Ammerpohl

Thomas Gazlig born in 1966, degree in Biology focused on biochemistry, biotechnology and genetics (1987-1993, Technical University of Braunschweig) and post graduate degree in Journalism (1993-1996, Institute for Journalism and Communications Research at the University for Music and Theatre Hanover). Work experience: PR consultant in the Public Relations Department of the Ministry of Lower Saxony for Science and Art (1994-1995), and the insurance company Öffentliche Versicherung at Braunschweig (1995-1998). Since September 1998 press spokesman and head of the Public Relations Department of the GBF.

Figs: Analysis of the structures of the complex between Internalin A from Listeria monocytogenes *and human E-Cadherin (le); Dr. Wolf-Dieter Schubert mounting a protein crystal on an X-ray station (ce); Working under a clean bench is a precondition for many steps to get reliable results (ri).*

Photos: Bierstedt

SCIENTIFIC REPORTS INNOVATION REPORT

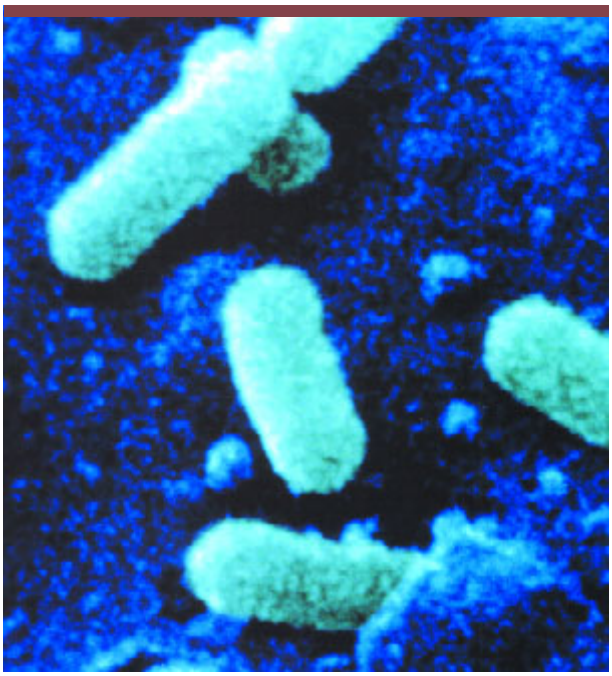
A Detailed Picture of the Interaction between Man and Bacteria

AUTHOR | **Prof. Dr. Dirk Heinz** | **Department of Structural Biology**

- Severe bacterial infectious diseases such as tuberculosis, whooping cough and dysentery are coming back into the focus of health research, even in the industrialized nations, due to the emergence of bacterial resistance to antibiotics, global tourism and poor vaccination schemes. At the same time, the enormous progress in the genome and proteome analyses of pathogenic bacteria and man opens up new possibilities for a more directed approach to research on infectious diseases.
Of special interest is the understanding of the complex interactions between bacteria and man at the molecular level that allow focussed and early intervention in the infection process. A potential weak point is the ability of many pathogenic bacteria to penetrate into the host cell where they continue the infection process from a niche which is well protected from the host's immune defence system. We aim to elucidate the high resolution structures of bacterial and human proteins involved in the respective infection processes. The structures of these macromolecules will form a rational basis for the development of new drugs and vaccines to fight or prevent bacterial infections.

The human pathogen *Listeria monocytogenes*

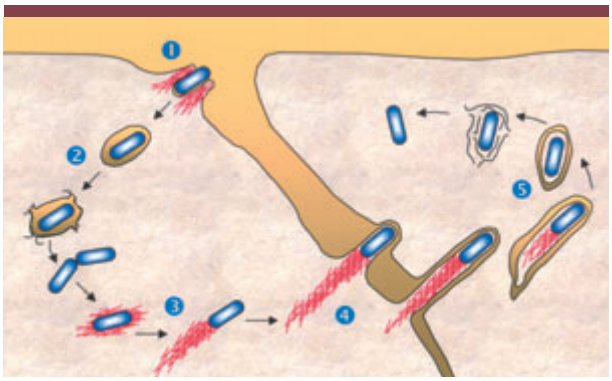
Listeria monocytogenes is a human pathogenic bacterium, which enters the host through food contamination. Infection mainly occurs in people with a compromised immune system and in pregnant women, where it can lead to a serious disease called listeriosis. In its acute form, life-threatening meningitis and meningoencephalitis can develop. The Gram-positive bacterium is able to breach three essential barriers in the human body: the intestinal wall, the placenta and the blood-brain barrier. Over the past few years *L. monocytogenes* has become an accepted model system for the study of facultative intracellular bacteria in infection biology.



- *Electron micrograph of* Listeria *adhering to human intestinal cells.*

Photo: GBF

During infection, the bacterium enters into the host cell, thus becoming "invisible" for antibodies of the humoral immune defence system. Within the host cell it can spread and multiply and subsequently infect neighbouring cells. For this purpose, the bacterium utilizes a limited set of proteins, so-called virulence factors, that cause a "reprogramming" of different host cell processes to the advantage of the bacterium. The protein family of internalins, for example, is responsible for host-cell specific adhesion and uptake of the bacteria by abusing several different host-cell signal transduction processes. Other important virulence factors include listeriolysin and two phospholipases, that free bacteria from membrane compartments following invasion, and ActA, which reorganizes the actin cytoskeleton to ensure mobility of the bacteria within the host cell.



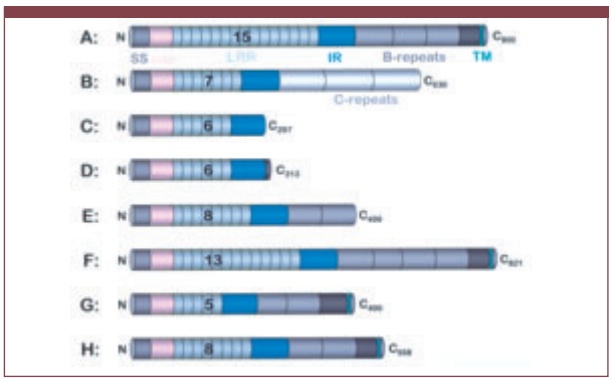
- *Infection cycle of* L. monocytogenes *(blue). Shown is the spreading of the bacteria in the host cells (beige).*

Internalins as keys to enter the host cell

Listeria harbour at their surface numerous proteins, including the protein family of internalins. By specifically contacting receptor molecules at the surface of host cells, internalins induce uptake of the entire bacterium by the host. This process, which resembles phagocytosis, requires major rearrangements of the host cell's cytoskeleton and is thus called "induced phagocytosis".

Internalin A (InlA), which is responsible for the uptake of *Listeria* by the intestinal epithelium, was discovered in 1991. Shortly afterwards, InlB was identified, which is required for the invasion of other host cells, such as hepatocytes and macrophages. Subsequently, additional "internalin-related" proteins were identified, that are mainly located at the bacterial surface – like the internalin InlH, whose function has yet to be characterised – or which are secreted by the bacteria. Their precise function, however, is unknown, although most of these proteins are present only in pathogenic *Listeria* strains, suggesting a potential role in the infection process. Charactistic to all proteins belonging to the internalin family is a modular organization of homologous stretches of amino acids, so called domains.

InlA is the largest member of the internalins, containing 800 amino acids. At its N-terminus it contains a short signal sequence, that allows export of the protein to the surface of the bacterium. The central part of the molecule contains two repetitive sequence elements, that are separated by a "inter repeat" – IR – sequence. At the C-terminus is a short LPxTG sequence motif, that is needed for covalently anchoring of the protein at the bacterial surface, followed by a hydrophobic, transmembrane α-helix and a charged cytoplasmatic tail. In the mature protein these two regions are absent.



- *Representative schematic of the amino acid sequences of different internalins. The individual domains are shown in different colours.*

The most characteristic feature of all internalins is the first repetitive element, which in the case of InlA consists of 15 repeating units each 22 amino acids in length. Due to the periodic accumulation of leucines or leucine-like amino acids, this repeat is called a "leucine rich repeat" (LRR). The consensus sequence of the internalin LRR is xxLxLxxNxLxxLxxLxxLxxL, where L and N stand for leucine (or related aliphatic amino acids) and asparagine, respectively, and x for any amino acid. LRR-domains are frequently found in eukaryotic proteins, where they are ideally suited for specific interaction with other proteins. In all internalins, the LRR-domain is flanked by a cap and the IR-sequence, which are both highly conserved.

InlB, which consists of 630 amino acids, is also associated with the bacterial surface. In contrast to the peptidoglycan anchor of InlA, the cell wall binding region comprises the C-terminal 232 amino acids. At its N-terminus, InlB also contains a LRR-sequence that consists of seven LRRs.

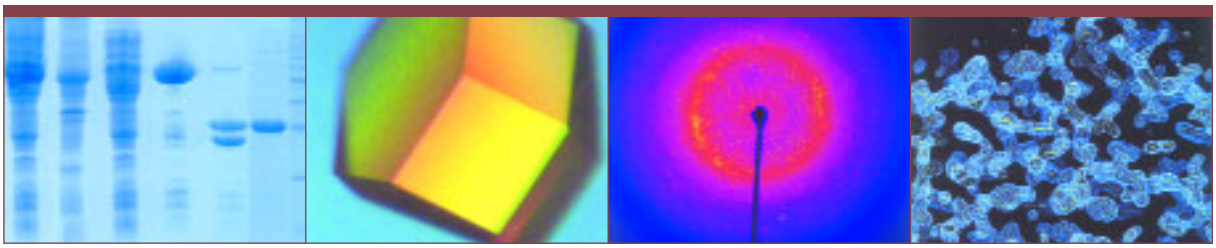
Invasion studies with truncated InlA- and InlB-proteins have shown that the region comprising the cap-, LRR- and IR-domains is sufficient for host cell specific invasion by the bacterium. Latex beads coated with these truncated proteins were also taken up by the host cells. The characteristic feature of LRR-domains to act as protein-protein interaction modules therefore suggests that the LRR-domains in internalins are utilized to interact with host cell receptors.

Human internalin receptors In 1996, human E-cadherin, a surface protein of the intestinal epithelium responsible for the tight connection of gut cells, was identified as the receptor of InlA. E-cadherin belongs to the superfamily of cadherins, that recognize their peers on neighboring cells via homophilic contacts, leading to cell associations in different tissues.

InlB shows specific interactions with glycosaminoglycans and binds to two different receptors: gC1-qR, a protein of the complement system, and Met, a transmembrane receptor tyrosine kinase. The physiological agonist of Met is hepatocyte growth factor (HGF), that is responsible for a multitude of important processes, such as cell growth, wound healing and tumour metastasis. Met-activated signal transduction processes mainly lead to a reorganisation of the actin cytoskeleton. A number of effects that are caused by HGF, such as the scattering of cells, are also stimulated by InlB.

To increase our understanding of the function of the internalins and Listerial invasion at the atomic level, we have elucidated the 3D-structures of these proteins. over the past four years. This work has been done in close cooperation with the groups of Trinad Chakraborty at the University Gießen and Jürgen Wehland from the GBF. Besides the structures of the receptor binding domains of InlA, InlB and InlH, whose function has not yet been characterised, we were able to solve the 3D-structure of the complex between the LRR-domain of InlA and the N-terminal domain of the human receptor E-cadherin. In the following, the LRR-domains are called InlA', InlB' and InlH', in contrast to the complete protein.

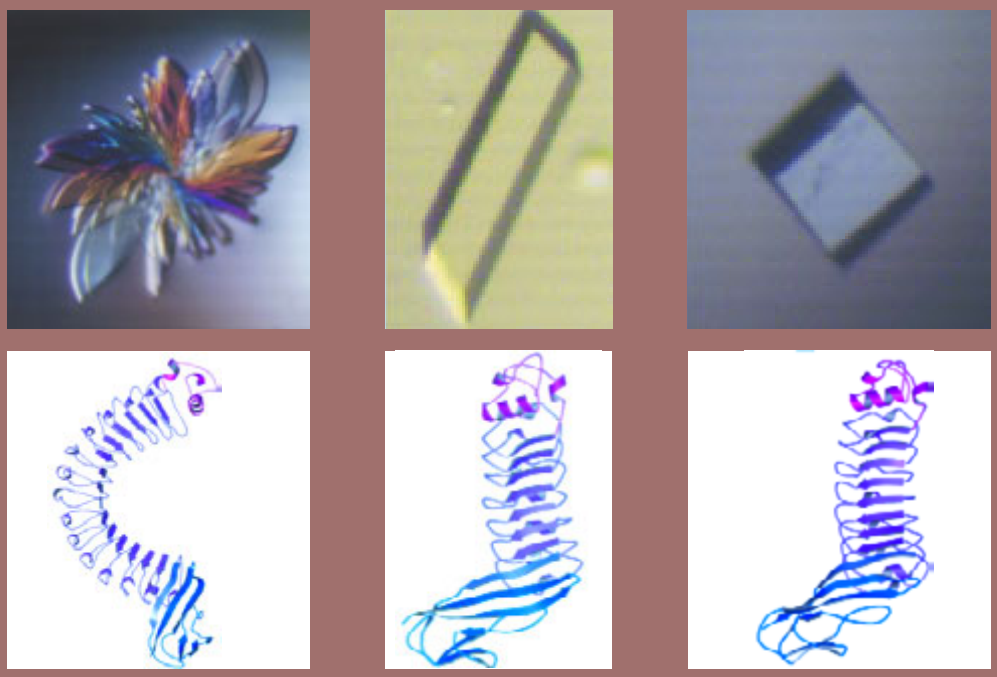
Structural analysis of proteins Proteins play a pivotal role in nearly all processes relevant for life. Due to its characteristic and spatially defined 3D-structure, each protein is optimized for its physiological function. Elucidation of the 3D-structure, *i.e.* the precise location of thousands of atoms in the macromolecule, still represents a challenging task. The method of choice for proteins with a molecular mass larger than 30 kDa is X-ray structure analysis: This method requires the crystallization of proteins from pure and homogeneous protein solutions. Unfortunately, successful protein crystallization is often very difficult, making the process often time-consuming and tedious. Once obtained, the small protein crystals are exposed to monochromatic X-rays, giving rise to diffraction images with complex geometries, from which the atomic coordinates (*i.e.* the electron density) of the protein can be calculated.



● *From the purified protein to its crystal structure*

Photo: GBF

Crystal structures of internalins Over a number of years, we have been able to produce well diffracting crystals of InlA', InlB' and InlH' and subsequently solved the high resolution structures of the recombinant proteins. As expected from their amino acid sequences, the internalins show a modular architecture. The structures of InlB' and InlH' consist of a central LRR-domain that is flanked by a smaller N-terminal cap-domain and a C-terminal immunoglobulin-like IR-domain.



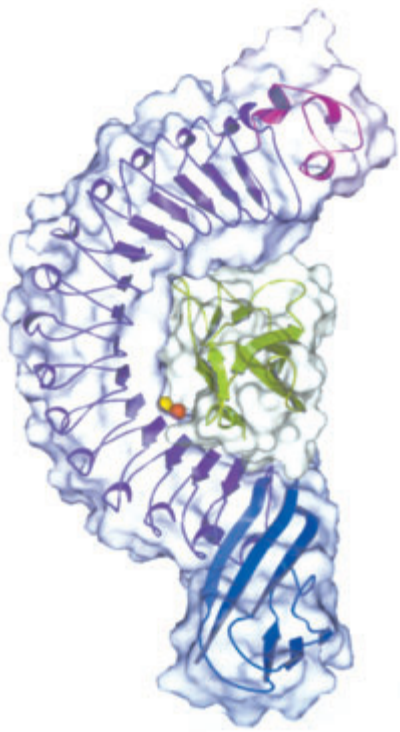
- *Crystals and inferred structures of InlA', InlB' and InlH'. The structures consisting of cap- (pink), LRR- (magenta) and IR-domain (blue) are depicted as ribbon diagrams showing the respective secondary structural elements (helices, loops and β-strands).*

Photo: GBF

In the case of InlB', the LRR-domain consists of seven LRRs, each composed of a β-strand and a tightly wound 3_{10}-helix connected via loops. In the slightly curved 3D-structure of the domain, the solenoid-like arranged LRRs are stacked, leading to a parallel β-sheet forming the inner concave side and stacked helices defining the outer convex surface. The leucines and leucine-like amino acids pointing towards the centre of the spiral, constitute the hydrophobic core of the domain. Therefore, they must fulfil a solely structural function by stabilizing the domain. The neighbouring cap- and IR-domains shield this hydrophobic core from the surroundings, thus providing additional stability to the LRR-domain. While InlH' with eight LRRs and InlB' with seven LRRs closely resemble each other, InlA' with 15 LRRs shows a much more pronounced curvature. Together with the C-terminal IR-domain, the structure of InlA' has a sickle-shaped appearance.

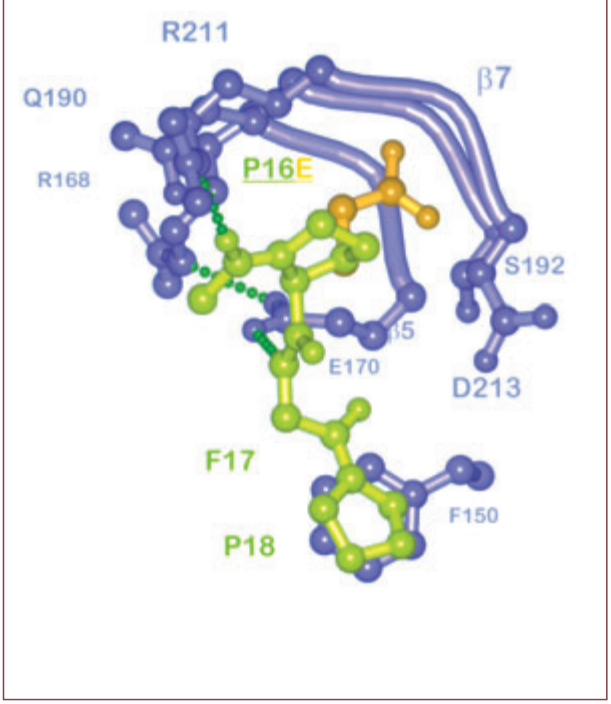
Structure of an internalin/receptor complex

Recently, we were successful in determining the crystal structure of the stoichiometric complex between InlA' and the N-terminal domain of the human receptor E-cadherin hEC1. This structure provided, for the first time, a high resolution picture of the initial step of *Listeria* infection: the adhesion of the bacteria to the human intestinal wall.



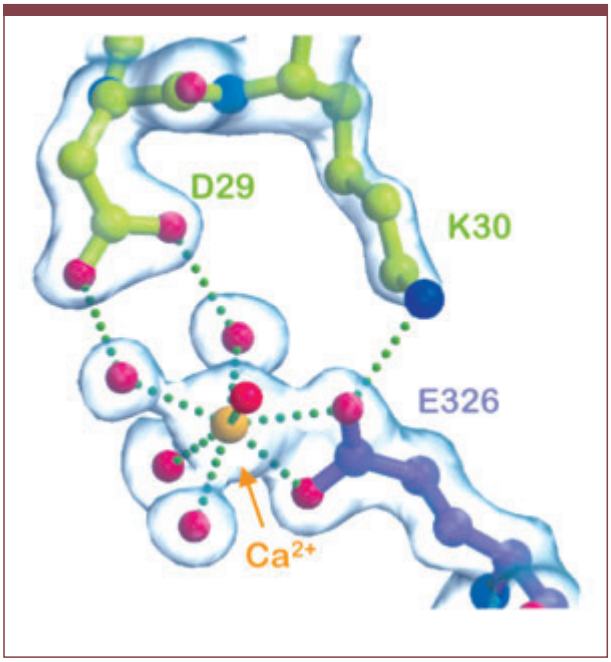
- *Structure of the complex between InlA' and hEC1 (green). The ribbon diagrams are superposed on the surface representation of both molecules (grey). Calcium and chloride ions at the interface between both proteins are shown as coloured spheres.*

In the structure of the complex, InlA' surrounds the smaller hEC1 domain almost completely. The C-termini of both proteins point in opposite directions, an absolute necessity for an as-close-as-possible contact between host and bacterial cells. Numerous, predominantly hydrophobic amino acids contribute to the highly complementary recognition between both proteins. An interesting feature is the participation of nearly all LRRs of InlA' in the interaction with hEC1. Of special interest is a proline residue located at position 16 in hEC1, which fits perfectly into a hydrophobic pocket at the surface of InlA'.



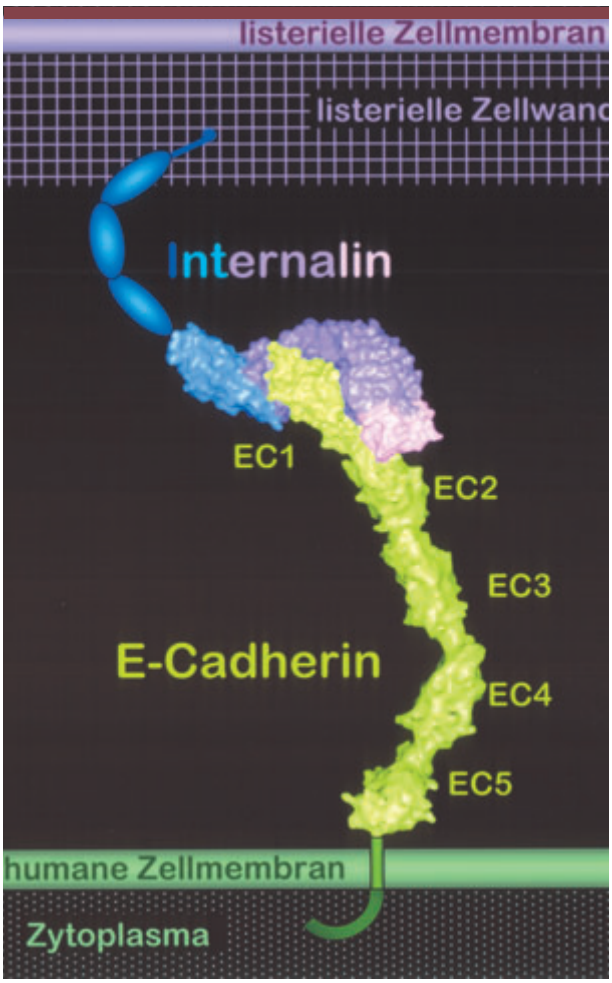
- *Interaction between proline 16 of hEC1 (green) and InlA' (magenta). Superimposed is the* in silico *mutation of this amino acid to glutamic acid (corresponding to murine E-cadherin which would lead to steric and electronic clashes.*

In the murine E-cadherin, this residue is replaced by glutamic acid, which, due to its negative charge and size, is not able to properly interact with InlA. Therefore, the structure of the complex provides an impressive molecular explanation for the observation that mice are insensitive to orally administered *Listeria*. Another interesting feature is the apparent weakness of the complex. Binding studies using the analytical ultra-centrifuge – in cooperation with Claus Urbanke, MHH – show a dissociation constant in the micromolar range. For strong protein-protein interactions this value is usually orders of magnitude smaller. Despite this relative weak interaction between both proteins, bacteria can nevertheless adhere to the host cell. During adhesion, several complexes that form gradually, similar to a zipper, presumably support each other in a cooperative fashion. In addition, the interaction between both proteins is modulated by a regulatory calcium ion located at the interface.



- *The Ca^{2+}-binding site at the interface between InlA' (magenta) and hEC1 (green).*

Following invasion, dissociation of the metal ion in the cytoplasma of the host cell presumably facilitates detachment from the host cell membrane, allowing the unhindered spread of the bacteria in the host cell. A schematic model of the interactions between *L. monocytogenes* and human intestinal cells, based on the crystal structure of the complex, is shown below. This figure illustrates how InlA grips E-cadherin like a hook mediating the adhesion of the bacteria to the intestinal wall. Several of these simultaneously formed complexes lead to adhesion of the bacterium sufficiently strong enough to initiate host-cell processes that lead to its uptake.



- *Model of the InlA-mediated adhesion of* L. monocytogenes *(magenta) to the human intestinal epithelium (green). Shown are the surface representations of the structures of InlA' and the extracellular domains of E-cadherin.*

Interaction of InlB and Met Using site-directed mutagenesis, we were recently able to show that the interaction between InlB and Met occurs, similar to InlA, via the concave surface of the LRR-domain of InlB. This region of the structure of InlB' has an array of aromatic amino acids that extend along the entire LRR-domain. By replacing these amino acids with polar amino acids, we could show that they are in fact critical for the bacterial invasion of host cells. Hence, a precise knowledge of the structure of internalins allows us to "reduce" the invasion of *Listeria* to a few amino acids that are critical for the infection process.

Conclusions and perspectives As exemplified by our study of the internalins, the techniques of modern structural biology allow the precise detection of target points for a specific intervention in infection processes for the development of new strategies against bacterial infection. Bacterial virulence factors, through their mimicry of host cell processes, can also be used as tools to better understand these processes. Based on the structure of the InlA'/hEC1-complex, we plan to design InlA'-mutants which show a much higher affinity towards E-cadherin. These mutants could be used to investigate hitherto poorly understood E-cadherin signalling in the host cell. To study Listerial infections in the mouse model, the design of an InlA'-variant able to recognize murine E-cadherin would be very helpful. As latex beads coated with internalins are efficiently taken up by host cells, it is plausible to use the internalins as "vehicles" to transport drugs to specific target cells, such as cancer cells.

- *Upper row, from left to right: Joop van den Heuvel, Guido Dieterich, Hartmut Niemann, Wolf-Dieter Schubert, Karsten Bruns, Joachim Reichelt, Ilse Padrock (hidden), Detlef Hanisch, Hans-Jürgen Hecht and Dirk Krumme Lower row, from left to right: Victor Wray, Rita Getzlaff, Steffi Ehinger, Beate Jaschok-Kentner, Marina Lindemann, Sabine Weißflog, Christel Kakoschke, Daniela Gebauer, Andrea Abrahamik, Susanne Frese, Claudia Hanko, Manfred Nimtz and Dirk Heinz.*

Photo: Bierstedt

Dirk W. Heinz born in 1960, studies in Chemistry (1980 – 1986, University of Freiburg), PhD in Biochemistry (1986 – 1990; University of Basel), Postdoctoral Fellow (1990 – 1993, University of Oregon, Eugene, U.S.A.), Research fellow (Habilitand) (1993 – 1998, University of Freiburg), Habilitation in Biochemistry (1998), Head of Junior Research Group at GBF (1998 – 2002). Since 2002 Head of the Department of Structural Biology a the GBF, since 2003 extraordinary professor at the Technical University Braunschweig.

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LITERATURE Listed are our own publications relating to *Listeria* infection, as well as selected papers from other groups.

- *Moser, J., Gerstel, B., Meyer, J. E. W., Chakraborty, T., Wehland, J. & Heinz, D. W. (1997). Crystal structure of the phosphatidylinositol-specific phospholipase C from the human pathogen* Listeria monocytogenes. *J. Mol. Biol. 273, 269-282.*
- *Heinz, D. W., Essen, L.-O. & Williams, R. L.(1998). Structural and mechanistic comparison of prokaryotic and eukaryotic phosphoinositide-specific phospholipases C. J. Mol. Biol. 275, 635-650.*
- *Schubert, W.-D., Göbel, G., Diepholz, M., Darji, A., Kloer, D., Hain, T., Chakraborty, T., Wehland, J., Domann, E., Heinz, D.W. (2001) Internalins from the human pathogen* Listeria monocytogenes *combine three distinct folds into a contiguous internalin domain. J. Mol. Biol. 312, 783-794.*
- *Machner, M. P., Urbanke, C., Barzik, M., Otten, S., Sechi, A. S., Wehland, J. & Heinz, D. W. (2001). ActA from the human pathogen* Listeria monocytogenes *is a monomer simultaneously interacting with four Ena/VASP homology 1 domains. J. Biol. Chem. 276, 40096-40103.*
- *Heinz, D. W. (2002). Modellsystem für Infektionen – Pathogene Bakterien auf ihrem unheilvollen Weg verfolgt. Jahresheft der Helmholtz-Gesellschaft. pp. 10-11.*
- *Schubert, W.-D., Urbanke, C., Ziehm, T., Beier, V., Machner, M. P., Domann, E., Wehland, J., Chakraborty, T. & Heinz, D. W. (2002). Structure of internalin, a major invasion protein of* Listeria monocytogenes, *in complex with its human receptor E-cadherin. Cell 111, 825-836.*
- *Machner, P., Frese, S., Schubert, W.-D., Orian-Rousseau, V., Niemann, H, Wehland, J. & Heinz, D. W. (2003). Aromatic amino acids at the surface of InlB are essential for host cell invasion by* Listeria monocytogenes. *Mol. Microbiol. 48, 1525-1536.*
- *Schubert, W.-D. & Heinz, D.W. (2003). Structural aspects of adhesion and invasion of host cells by the human pathogen* Listeria monocytogenes. *ChemBioChem., in press.*
- *Bierne, H., and Cossart, P. (2002) InlB, a surface protein of* Listeria monocytogenes *that behaves as an invasin and a growth factor. J Cell Sci 115: 3357-3367.*
- *Boggon, T.J., Murray, J., Chappuis-Flament, S., Wong, E., Gumbiner, B.M. and Shapiro, L. (2002). C-Cadherin ectodomain structure and implications for cell adhesion mechanism. Science 296, 1308-1313.*
- *Braun, L., and Cossart, P. (2000) Interactions between* Listeria monocytogenes *and host mammalian cells. Microbes Infect 2: 803-811.*
- *Cossart, P. and Lecuit, M. (1998). Interactions of* Listeria monocytogenes *with mammalian cells during entry and actin-based movement: bacterial factors, cellular ligands and signaling. EMBO J. 17, 3797-3806.*
- *Daniels, J.J.D., Autenrieth, I.B. and Goebel, W. (2000). Interaction of* Listeria monocytogenes *with the intestinal epithelium. FEMS Microbiol. Lett. 190, 323-328.*
- *Finlay, B.B., and Cossart, P. (1997) Exploitation of mammalian host cell functions by bacterial pathogens. Science 276: 718-725.*
- *Gaillard, J.L., Berche, P., Frehel, C., Gouin, E. and Cossart, P. (1991). Entry of* L. monocytogenes *into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. Cell 65, 1127-1141.*
- *Galan, J.E. (2000). Alternative strategies for becoming an insider: Lessons from the bacterial world. Cell 103, 363-366.*
- *Garandeau, C., Réglier-Poupet, H., Dubail, I., Berezzi, J.-L., The European* Listeria *Genome Consortium, Berche, P., Charbit, A., (2002) The sortase SrtA of* Listeria monocytogenes *is involved in processing of internalin and in virulence., Infect. Immun. 70, 1382-1390.*
- *Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., et al. (2001). Comparative genomics of* Listeria *species. Science 294, 849-852.*
- *Glomski, I.J., Gedde, M.M., Tsang, A.W., Swanson, J.A. and Portnoy, D.A. (2002) The* Listeria monocytogenes *hemolysin has an acidic pH optimum to compartmentalize activity and prevent damage to infected host cells. J. Cell Biol. 156, 1029-1038.*
- *Kobe, B. and Kajava, A.V. (2001). The leucine-rich repeat as a protein recognition motif. Curr. Opin. Struct. Biol. 11, 725-732.*
- *Lecuit, M., Dramsi, S., Gottardi, C., Fedor-Chaiken, M., Gumbiner, B. and Cossart, P. (1999). A single amino acid in E-cadherin responsible for host specificity towards the human pathogen* Listeria monocytogenes. *EMBO J. 18, 3956-3963.*
- *Lecuit, M., Vandormael-Pournin, S., Lefort, J., Huerre, M., Gounon, P., Dupuy, C., Babinet, C. and Cossart, P. (2001). A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. Science 292, 1722-1725.*
- *Marino, M., Banerjee, M., Jonquieres, R., Cossart, P. and Ghosh, P.(2002). GW domains of the* Listeria monocytogenes *invasion protein InlB are SH3-like and mediate binding to host ligands. EMBO J. 21, 5623-5634.*
- *Mengaud, J., Ohayon, H., Gounon, P., Mege, R.M. and Cossart, P. (1996). E-cadherin is the receptor for internalin, a surface protein required for entry of L. monocytogenes into epithelial cells. Cell 84, 923-932.*
- *Parida, S.K., Domann, E., Rohde, M., Muller, S., Darji, A., Hain, T., et al. (1998) Internalin B is essential for adhesion and mediates the invasion of* Listeria monocytogenes *into human endothelial cells. Mol Microbiol 28: 81-93.*
- *Schlech, W. F. (2000). Foodborne listeriosis. Clin. Infect. Dis. 31, 770-775.*
- *Shapiro, L., Fannon, A.M., Kwong, P.D., Thompson, A., Lehmann, M.S., Grubel, G., Legrand, J.F., Als-Nielsen, J., Colman, D.R., Hendrickson, W.A. (1995). Structural basis of cell-cell adhesion by cadherins. Nature 374, 327-337.*
- *Shen, Y., Naujokas, M., Park, M., and Ireton, K. (2000) InIB-dependent internalization of* Listeria *is mediated by the Met receptor tyrosine kinase. Cell 103: 501-510.*

New Improved Vaccines to Prevent Human Diseases

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Impact of infectious diseases

Infectious diseases have a tremendous impact on human health. Approximately one third of all deaths occurring each year worldwide are caused by infectious agents. Microorganisms are also responsible for at least 15% of cancers, such as gastric cancer, hepatocarcinoma and cancer of the cervix, and are also implicated in the pathogenesis of many chronic diseases, such as neurological disease, inflammatory disease or cardiovascular disease. In addition, infections are usually the final cause of death in individuals afflicted by a non-infectious primary disease, such as trauma or chronic obstructive pulmonary disease. The importance of infectious disease is actually increasing because of the emergence of new pathogens, such as HIV, *Helicobacter pylori*, Hanta virus or HCV, and because of the re-emergence of diseases that were considered to be under control (e.g. diphtheria and tuberculosis). Tropical diseases are also spreading to new areas, as a result of global warming and increased mobility. Finally, aging individuals and immune-suppressed patients are particularly susceptible to opportunistic pathogens.

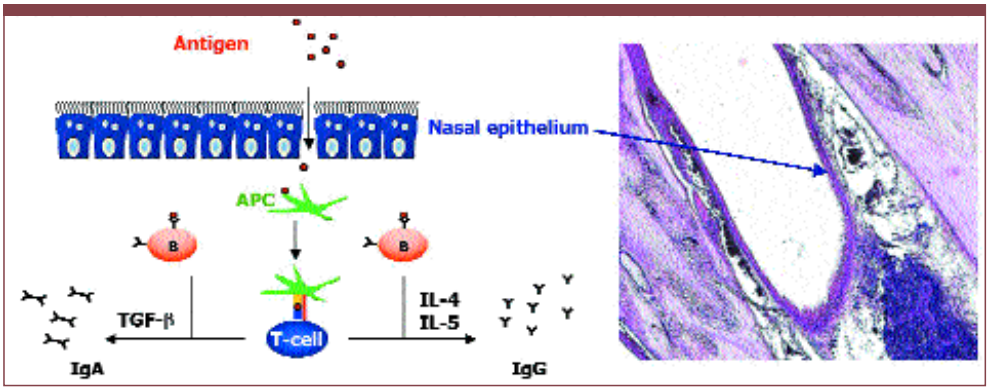
Vaccines for prevention and therapy Prevention and therapy are the two complementary approaches for tackling the problem of infectious disease. However, the clinical management of infected patients is rendered more difficult by the worldwide emergence of multi-drug resistant strains. Furthermore, a disproportionate reliance on treatment implies the tacit acceptance of the human suffering and potential consequences associated with disease. Furthermore, the high direct costs of patient care, as well as escalating indirect costs, are not prevented by treatment. Thus, it is essential to strengthen our efforts to develop efficient prophylactic interventions. The main strategies used to prevent infectious disease focus on the protection of susceptible individuals and prevention of pathogen spreading. This can be achieved by hygiene measures, such as improvement of water quality, reservoir and vector reduction and quarantine of infected individuals, or by vaccination, which is the most cost-efficient intervention.

Although vaccines have generally been used to prevent infectious diseases, the possibility of using them also therapeutically is gaining interest. Boosting of the immune system of infected individuals by active immunization – alone or in combination with conventional therapy – may lead to improved cure rates or to a shortened period of treatment. Vaccines can also be used for the immunotherapy and immunoprophylaxis of cancer and for a wide range of non-infectious chronic diseases, as well as for birth control in humans and animals. However, there are still many diseases for which vaccines are not yet available, or for which the available vaccines are not completely satisfactory in terms of efficacy, stability or cost. Thus, there is an urgent need for both new and improved vaccines.

Mucosal vaccines Most infectious agents are either restricted to the mucosa or need to transit it during the early steps of the infection. Therefore, the elicitation of an efficient immune response at the location where the first line of defense is laid is highly desirable.

Advantages associated with mucosal vaccination

- High acceptance
- Increased compliance
- Reduced side effects
- Stimulation of systemic and mucosal immune responses
- Reduction of microbial colonization
- Impairment of microbial transmission to susceptible hosts
- Simple administration logistics
- Low delivery costs



- *Mucosal activation of B cells. Antigens are taken-up by APC, processed and presented on MHC II molecules to T cells. Activated T cells provide help to antigen-specific B cells and modulate the elicited responses. Secretion of IL-4 and IL-5 leads to IgG 1 production, whereas TGF-ß stimulates isotype switch towards IgA and IgG 2b. Secretory IgA plays an important role in the mucosal immune system, by neutralizing pathogens directly at the port of entry.*

The stimulation of a pathogen-specific response at the entry site is expected to impair infection, *i.e.* colonization, thereby reducing the risk of transmission to susceptible hosts. Parenterally administered vaccines mainly stimulate systemic responses, whereas vaccines administered by the mucosal route mimic the immune response elicited by natural infections, thereby leading to efficient mucosal and systemic responses. Furthermore, the existence of a common mucosal immune system allows stimulation of an immune response at mucosal effector sites remote from the vaccination site. However, there is a certain level of compartmentalisation, which leads to variations in response at different effector sites. Use of the mucosal route is also in itself associated with a considerable number of advantages.

Unfortunately, antigens administered by this route are usually poorly immunogenic. This is in part due to rapid antigen clearance, degradation by local enzymes, poor penetration, and the presence of a local tolerogenic milieu. Thus, different strategies have been exploited to increase the immunogenicity of antigens delivered by the mucosal route, such as their expression by live attenuated bacterial or viral carriers; their incorporation in physical or biological particles, liposomes, immune stimulatory complexes (ISCOM) or virosomes; their expression in transgenic plants and their co-administration with mucosal adjuvants.

However, antigen delivery is not sufficient *per se* to engender a protective response. It is also essential to stimulate the appropriate quality of immune response, *e.g.* antibodies or cell-mediated immunity. Thus, a successful vaccination strategy demands the right choice of adequate antigens, as well as their appropriate delivery and formulation. In this context, recent studies have demonstrated the possibility of eliciting desired immune responses at systemic and mucosal levels by combining different strategies or technology platforms for antigen delivery in prime-boost vaccination protocols.

Live carriers as a delivery system for protein- and DNA-based vaccines Both attenuated and commensal microorganisms have been exploited as carriers for vaccine antigens. The use of commensals is appealing because of their excellent safety profile and the possibility of obtaining long-term local expression of the selected antigen. On the other hand, attenuated pathogens are also attractive, since protection against the pathogen itself and immune responses specific for the heterologous antigens can be achieved simultaneously. Bacteria have been attenuated by the introduction of deletions in genes that are essential for either their virulence or their metabolism – for example, the synthesis of cell wall components, DNA or essential metabolites. The introduction of several independent attenuating deletions makes the risk of reversion to virulence as a result of recombination events negligible. Furthermore, mutations that are even safe in immune compromised hosts have been identified. The use of bacterial carriers is associated with all the general advantages of mucosal vaccination. In addition, their production is simple and cost-efficient, there are fewer restrictions in terms of storage and cold-chain maintenance, and the delivery costs are low. All the properties associated with the use of live vectors make this vaccination approach particularly suitable for mass immunization campaigns, especially in developing countries.

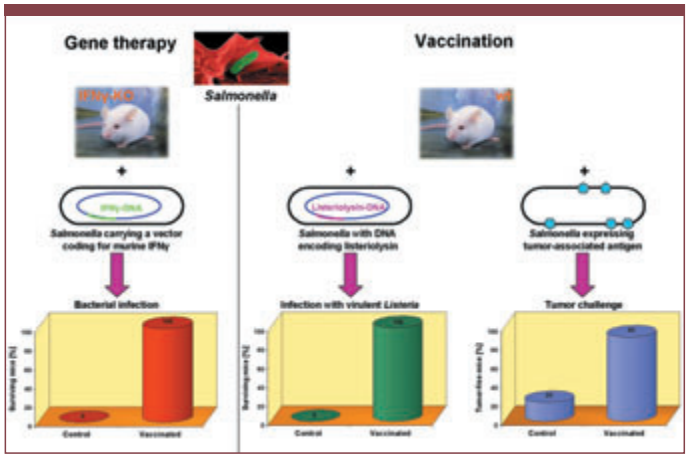
Recent studies have shown that bacterial vectors can also be used as a delivery system for so-called "DNA vaccines". Instead of using purified antigens, individuals are vaccinated with eukaryotic expression vectors coding for the selected antigens. In this case, the biosynthetic machinery of the vaccinee's own cells is responsible for antigen production. However, traditional naked DNA vaccination is extremely inefficient, because multiple administrations and high dosages are needed.

Properties of live vaccines

- Replicate in the host
- Limited number of doses are needed
- No adjuvants are needed
- Delivered by natural infection route
- Stimulate systemic and mucosal immune responses
- Variable safety in immune compromised individuals
- Easy production, transport and storage

The use of bacterial carriers as a delivery system eliminates the requirement for DNA purification and allows specific targeting of antigen presenting cells (APC). The carrier also acts as a natural adjuvant because of the presence of bacterial components, such as cell wall degradation products and unmethylated DNA, which promote recruitment of innate immunity masters and APC maturation through the activation of pattern recognition receptors. Thus, a local environment conducive to antigen presentation is specifically created.

It has been widely demonstrated that the use of bacteria as either conventional protein or DNA vaccine carriers can confer efficient protection against both infectious diseases and tumours. It has also been shown that bacteria-mediated gene delivery might represent an attractive alternative therapy for diseases in which the affected tissues are natural targets of the carrier microorganism, such as macrophages, dendritic cells or hepatocytes.



- *Attenuated* Salmonella *are attractive carriers for protein- or DNA-based vaccines. After oral vaccination with Salmonella carrying a plasmid coding for murine IFNg, IFNg-KO mice were protected against bacterial infections.* Salmonella*-mediated DNA vaccination was also able to confer protection against a lethal challenge with* Listeria monocytogenes*, whereas immunization with a carrier expressing a tumour-associated antigen prevented tumour take and reduced the number of lung metastasis after challenge with an aggressive murine fibrosarcoma.*

Physical and biological particles Antigen can be entrapped into physical particles, such as microspheres, virus-like particles or bacterial ghosts. When antigens are incorporated, either adsorbed or chemically bound to physical particles, more efficient immune responses can be stimulated, as a result of antigen protection against degradation, facilitated uptake by APC, and improved processing and presentation. Recombinant viral-like particles also constitute a new approach for vaccine development.

Recombinant DNA techniques make possible the insertion of foreign epitopes into proteins with inherent multimerization capacity – as viral capsid or envelope proteins – which, due to their highly symmetric structure and immunological properties, facilitate the stimulation of humoral and cellular responses against the inserted epitope.

The so called "bacterial ghosts" are a special type of particle. They are obtained by controlled expression of the PhiX174 gene E in Gram-negative bacteria. This protein forms a transmembrane tunnel through the bacterial envelope. Thus, bacterial ghosts have intact envelope structures, but are devoid of cytoplasm, which is expelled through the tunnel. They are specifically targeted to APC, in which they promote maturation through the provision of a potent danger signal by their structural components. Ghosts can not only be used as vaccines against diseases caused by the inactivated microorganisms, but also as a delivery system, since they can be loaded with antigens or, alternatively, recombinant antigens can be expressed before lysis.

Liposomes, ISCOMS and virosomes Liposomes have been extensively used as antigen delivery systems. They are lipid vesicles, formed when phospholipids are exposed to an aqueous environment. During this process any antigen present in the aqueous phase will be retained within the vesicle. Liposomes do not only protect the antigens present in the formulation, but also act as immunoadjuvants. ISCOMs are formed from cholesterol, lipid, immunogen and saponin, and constitute a related approach for both parenteral and mucosal vaccines. Finally, virosomes are unilamellar vesicles obtained by reconstitution of empty influenza virus envelopes, which are devoid of viral nucleocapsid and genetic material. Virosomes contain functional viral envelope glycoproteins, which stimulate both MHC I- and MHC II-restricted responses. The phagosomal pH shift results in conformational changes of the hemagglutinin, which trigger membrane fusion and virosome release into the cytoplasm. Antigens linked to the virosomal surface are partially proteolysed within the APC endosome, leading to MHC class II antigen presentation. On the other hand, MHC class I-restricted presentation is achieved upon antigen escape to the cytosol, as a result of the fusion activity of the carrier virosome. Virosomes also provide antigen protection against degradation, depot effect and a regular-repetitive virus-like particle structure.

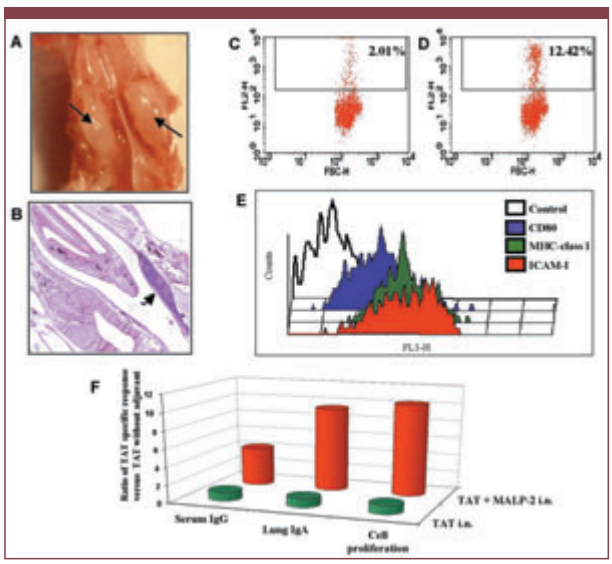
Transgenic plants Recombinant DNA technology has facilitated the introduction of a variety of genes into plants. The production of vaccine-relevant antigens in food plants like banana trees and potatoes enables direct mucosal delivery through consumption of the recombinant plants. The possibility of expressing vaccine antigens in seeds expands the potential uses of this approach, mainly in animal husbandry, facilitating both storage and conservation. Interestingly, this strategy can also be exploited for the development of immuno-contraceptive vaccines for herbivore species.

Mucosal adjuvants An alternative approach to the stimulation of efficient mucosal immune responses is based on the use of mucosal adjuvants. Simply mixing the antigen with mucosal adjuvant is the easiest and preferred method. Alternatively, the adjuvant can be covalently linked to the antigen. In this case, the adjuvant also acts as a carrier and stabilizing moiety. Mucosal adjuvants can also be combined with other mucosal delivery systems, such as live vectors, physical particles or lipo-somes. An interesting approach is the development of chimeric moieties in which adjuvant and targeting properties of different molecules are combined. This combinatory approach dramatically expands the possibilities for modulating the immune responses elicited using different mucosal antigen delivery systems.

Bacterial toxins, such as cholera toxin, the heat-labile toxin of *Escherichia coli*, and their derivatives were the first molecules exploited for this purpose. However, their use in humans was limited by their toxicity. Therefore, non-toxic derivatives have been developed, which retain the adjuvanticity. Several components from Gram-positive and Gram-negative bacteria have also been used as adjuvants.

Among them, the non-toxic lipopolysaccharide–derivative, monophosphoryl lipid A, which exhibits potent immunostimulatory properties, as well as the muramyl dipeptide-derivatives MDP-Lys[18] and N-acetylglucosaminyl-N-acetylmuramyl dipeptide, which have improved bioavailability and decreased toxicity.

We have recently demonstrated that a *Mycoplasma fermentans*-derived synthetic lipopeptide, macrophage-activating lipopeptide MALP-2, significantly enhances cellular and humoral immune responses against antigens co-administered by either the parenteral or mucosal route. Functional studies also enabled us to establish that intranasal administration of MALP-2 promotes recruitment and maturation of APC at the level of the nasal associated lymphoid tissues.



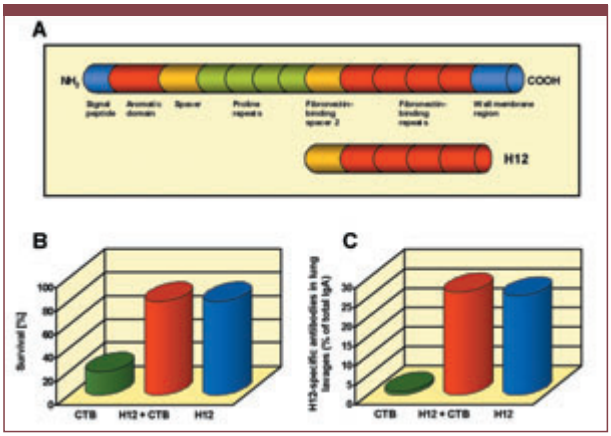
- *Efficient stimulation of nasal associated lymphoid tissues (NALT) using MALP-2 as mucosal adjuvant. The NALT are indicated by arrows in nasal cavities exposed after removing the upper palate (A) and in haematoxylin-eosin stained sections at 2.5x magnification(B). FACS analysis showed macrophage recruitment 16 h after intranasal administration of MALP-2, with 12.42% of MAC-1+ cells (D) compared to 2.01% in the controls (C). Macrophages also exhibit an up-regulated expression of MHC-class I, co-stimulatory (CD80) and adhesion (CD54) molecules (E). The local recruitment and activation of APC resulted in improved immune responses against co-administered vaccine antigens. Significantly improved cellular and humoral (both systemic and mucosal) immune responses against the HIV-1 Tat protein were observed after intranasal immunization with MALP-2 (F).*

Nasal vaccination with the HIV-1 Tat protein, co-administered with MALP-2, resulted in the elicitation of efficient humoral and cellular immune responses at the systemic level. In addition, efficient Tat-specific mucosal antibody responses were stimulated both at the inductive site of vaccination, as well as at distant mucosal sites, *e.g.* in the genitourinary tract. This demonstrates efficient migration and homing of the activated B cells.

Unmethylated CpG motifs, which are generally absent in mammalian DNA, also exhibit direct immunostimulatory effects on immune cells. Many base combinations with stimulatory activity have been exploited to improve the immune responses stimulated after vaccination by either the mucosal or parenteral route. On the other hand, it was also demonstrated that some anti-viral drugs, such as imidazoquinolines, enhance immune responses. A molecule obtained by linking the L-alanine-D-isoglutamine residue of muramyl dipeptide to amantadine – adamantylamide dipeptide – is an effective mucosal immunoadjuvant, with an adequate safety profile for human use.

We have recently shown that the fibronectin binding protein I (SfbI) from *Streptococcus pyogenes* is an efficient mucosal adjuvant able to substantially improve cellular, humoral and mucosal responses when coupled to or co-administered with an antigen. Although the use of this molecule promotes a dominant Th2 response, efficient cytotoxic T lymphocyte responses were also stimulated. Functional studies showed that SfbI promotes activation and maturation of APC, and that the fibronectin-binding domains are responsible for adjuvanticity. This interesting molecule is also a promising candidate vaccine antigen, since immunized animals are protected against lethal challenge with virulent *S. pyogenes*. Co-administration of a mucosal adjuvant is not required.

Thus, this protective vaccine antigen, with built-in adjuvant properties, is an attractive candidate for the development of multi-component vaccines.



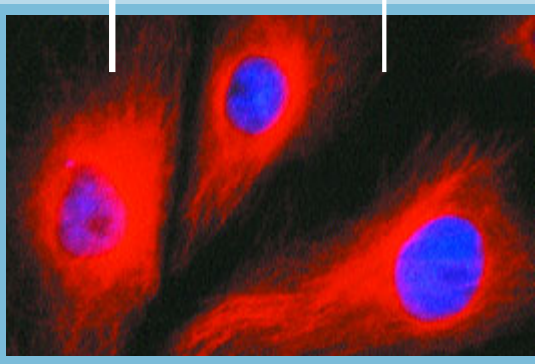
- *Fibronectin-binding protein I (SfbI): promissing vaccine candidate against* S. pyogenes *with built-in adjuvant properties.(A) Schematic structure of the SfbI protein and the recombinant derivative H12. (B) Mice intranasally immunized with the H12 fragment in the presence or absence of CTB were protected against lethal challenges with a virulent* S. pyogenes *strain. (C) Efficient SfbI-specific mucosal IgA responses were stimulated in mice.*

Outlook Vaccine development and use have facilitated efficient control of major human diseases. Despite their intrinsic efficacy, the first generation of vaccines was mainly designed on an empirical basis. However, the vast amount of knowledge gained in recent years in the fields of microbial pathogenesis, immunology and vaccinology facilitates the development of a new generation of well-defined and improved vaccines. These novel vaccines will exhibit an optimal safety profile and higher efficacy. The availability of novel antigen delivery systems, adjuvants and vaccination strategies will also enable fine-tuning of the elicited responses according to specific clinical needs. The use of the mucosal route of administration will be associated with higher acceptance and compliance. The stimulation of mucosal responses will also allow infections to be blocked at a very early stage, thereby breaking the transmission cycle by impairing microbial transfer to susceptible hosts. These developments are expected to be instrumental in achieving efficient prevention of human disease.

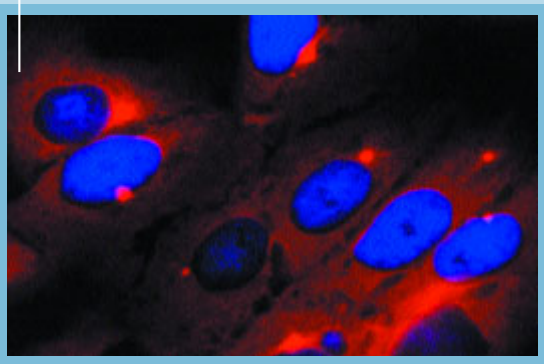
- *Faiza Rharbaoui, Thomas Ebensen, Claudia Link, Karina Watzke, Elena Reinhard, Urte Jäger, Kai Schulze, Carola de Domenico, Pablo Becker, Carlos A. Guzmán, Lothar H. Staendner, Axel Fey, Stefan Borsutzky and Karin-Heide Planck-Schumacher. (from left to right)*

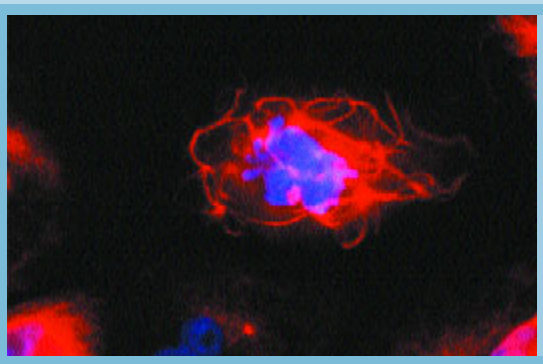
Photo: Schulze, GBF

Carlos A. Guzman born in 1959, Medicine studies (1976 – 1981, National University Rosario, Argentina), Specialisation in Bacteriology (1982 – 1986), Doctor in Medicine and Surgery (University of Genoa, Italy), Qualifying Medical Graduates Examination (1989, School of Medicine and Surgery, Genoa, Italy), Research Doctorate in Microbiological Sciences (1990 – 1993, University of Genoa, Italy), since 1994 Head Vaccine Research Group at the GBF, 2000 Habilitation "Venia Legendi" for Medical Microbiology (Hanover Medical School).



Microtubules (red) are part of the cytoskeleton of the cell (le). The tubulysins, which have been isolated from myxobacteria, induce a depletion of microtubules in the cell (ce). By chemical modification we try to find compounds that target selectively microtubules of dividing cells. In these cases, abnormal spindles are formed (ri), and cell propagation of tumor cells is inhibited (nuclei and chromosomes stained blue). Photos: GBF

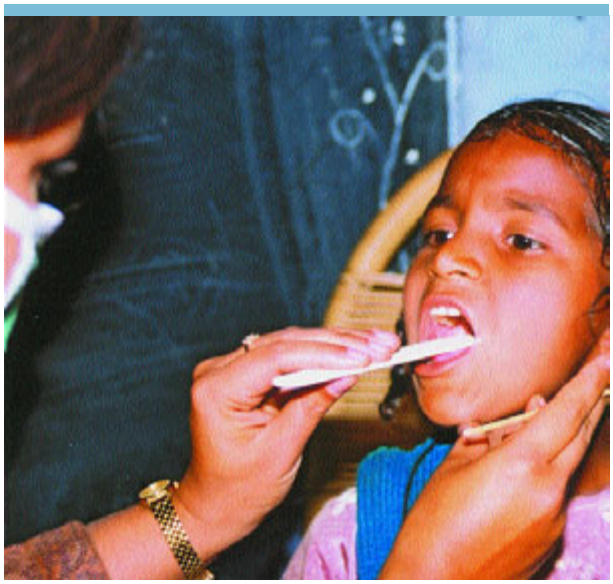




Programme "Infection and Immunity"

PROGRAMME SPEAKER | **Prof. Dr. Jürgen Wehland** | **Department of Cell Biology**

"The war against infectious diseases has been won." This statement, made in 1962 by the US Surgeon General, William H. Stewart, reflected the attitude of health care professionals and the public towards the medical importance of infectious disease at the time. There was a strong conviction that infectious diseases were no longer a threat, as had been the case just 50 years earlier. The successful development of antibiotics and vaccines had resulted in one of the greatest achievements in medical research. Eradication programmes for polio, measles and smallpox had created an atmosphere of security and contributed to the conviction that infectious diseases would soon be a problem of the past. As a result, public awareness, medical research and investments by the pharmaceutical industry in the development of new anti-microbial drugs diminished. There seemed to be little need to develop new antibiotics and vaccines or to invest in the field of infectious diseases. Germany, once a leader in vaccine development, discontinued almost all of its industrial activity in this area.

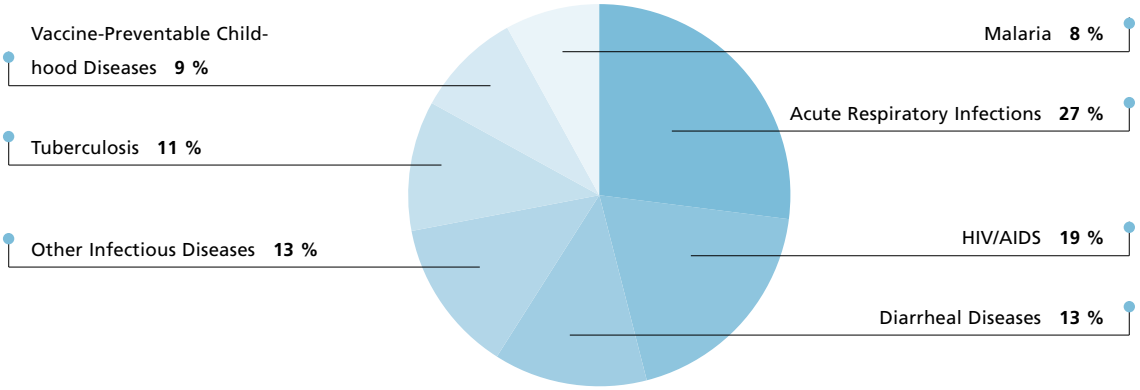


Medical examination of infectious diseases: Taking a smear from the throat of an Indian girl.

Photo: Gazlig, GBF

Alarming developments This view has changed dramatically, with more than seventeen million people still dying each year from infectious diseases – a third of all health-related deaths worldwide. While underdeveloped countries are most strongly affected, infectious diseases are also an increasing problem for industrialized societies. Newly emerging infections, such as HIV or SARS, have devastating effects and reach many countries through exchange of blood products or global travel. Environmental changes or alterations in food processing in developed countries allow new infectious agents to emerge, as revealed by the 'mad cow' epidemic. The number of re-emerging infectious diseases, once regarded as defeated, is rising as a result of antibiotic resistance. Multidrug resistance is developing against virtually all currently available drugs, for example in tuberculosis and malaria.

Deaths caused by infectious diseases, 2001



Source: WHO-Report 2002

Furthermore, opportunistic infections are a recurrent problem in immuno-compromised patients and an aging population. Despite these alarming developments, the opportunities for establishing new diagnostic and effective therapeutic strategies are better than ever. Systematic genome research is providing information on potential drug targets, thus aiding the development of new antibiotics. A better understanding of the functions of individual genes, combined with knowledge about the interactions of microbial genomes with host cellular genes will provide an excellent basis for the directed design of chemotherapeutic strategies against microbes. Functional genome analysis also provides insight into the molecular basis of immune responses and the genetic susceptibility to infectious diseases.

Equally important, our current knowledge of the molecular and cellular components of the immune system has opened up new possibilities of clinical intervention in the form of immunotherapies that extend beyond prophylaxis to therapeutic intervention. Today, our understanding of immunity extends far beyond its protective role against infectious diseases. The immune system not only protects the host from microorganisms but is also implicated in detecting altered cellular antigens. The precise mechanisms by which the immune system is undermined by certain microorganisms are barely understood. Examples are latency and immune escape.

Infection and Immunity The GBF programme "Infection and Immunity" is covering basic research in the area of infectious disease and immunity. It is at the interface of these fields where we expect the greatest potential for the development of new drugs and strategies to prevent and treat disease. The main objective of the programme is to understand the principle mechanisms that underlie the development of infectious diseases.

This involves basic research on model microorganisms and their pathogenicity, as well as a detailed analysis of the mechanisms of immunity. The aim is to understand the individual molecular and cellular steps that occur during the process of infection, the mechanisms by which selected microorganisms cause disease and the basic principles of the defence mechanisms used by the host to resist and control infections. This knowledge will be used to develop new tools to prevent and treat infectious diseases.

The topics of the research programme

- Microorganisms
- Pathogenesis
- Immunobiology
- Prevention and Therapy

Topic 01 – Microorganisms

TOPIC SPEAKER | **Prof. Dr. G. Singh Chhatwal** | **Department of Microbial Pathogenicity and Vaccine Research**

- Microbial infections are among the major causes of human disease. This topic deals with the molecular analysis of micoorganisms with the goal of identifying and characterising bacterial virulence factors and determining their structure-function relationship. The main focus will be on pathogenic streptococci and *Listeria*. In addition, probiotic *E. coli* Nissle 1917 and myxobacteria, as bacterial drug producers, will also be studied as part of this topic. Group A streptococci cause a wide range of diseases, which include invasive diseases, as well as sequelae, such as rheumatic heart disease. Pneumococci are capable of causing relatively harmless diseases, such as *Otitis media*, as well as life-threatening pneumonia and meningitis. Comparative genomics and proteomics studies as well as expression profiling will be performed to identify candidate genes encoding potential virulence factors in pathogenic streptococci. Furthermore, genes that are only present in certain sub-populations can be associated with specific clinical presentations. Particular emphasis will be placed on the identification of potential streptococcal rheumatogenic factors and bacterial proteins that interact with the extracellular matrix, or which are involved in bacterial adhesion, invasion and intracellular survival.

In addition, we aim to identify proteins that play an essential role in intracellular bacterial survival and motility, as well as cell-to-cell spreading, using *Listeria monocytogenes* as a model organism. In a first step, changes in expression pattern and post-translational modifications of membrane proteins under different physiological conditions will be characterized. Sequencing and characterization of novel biosynthetic pathways of another drug producing bacterium, *Sorangium cellulosum*, will also be studied in the framework of this topic.

Candidates which have been confirmed as virulence factors or potential drug targets will be studied further by investigating their 3D-structures using X-ray crystallography and NMR. The high resolution description of the 3D-structures of these proteins, or of relevant host-pathogen protein complexes, will allow the rational design of small molecule compounds that are able to interfere with their specific functions and thus provide a basis for the clinical development of novel therapeutics.

01.1 Genetic Variability of Streptococci

PROJECT LEADER | **Prof. Dr. G. Singh Chhatwal** | **Department of Microbial Pathogenicity and Vaccine Research**

PROJECT MEMBERS | Dr. Manfred Rohde | Dr. Rebecca Towers | Patricia Wegmeyer

Streptococci show large genetic variations among their virulence factors. These – and other – properties make them able to cause a wide spectrum of diseases in humans. The goal of this project is to determine the genetic variability of streptococci and its role in infection and sequelae, such as rheumatic fever. Streptococcal fibronectin-binding protein SfbI – also known as protein F1 – is involved in colonisation of epithelial tissues by *Streptococcus pyogenes* and is an important virulence factor. This protein is anchored on the cell surface and essential for adherence of the bacteria to the host. Comparative genetic analysis indicated a number of rearrangements in this gene locus which, therefore, represents a prominent site for gene transfer.

Evolution of SfbI In order to investigate mechanisms involved in the evolution of SfbI, the gene was sequenced from 54 different Streptococci-strains. Thirty-five distinct alleles were identified. Three principal mechanisms appear to have been involved in the evolution of SfbI: The amino-terminal aromatic-rich domain is the most variable region apparently generated by intergenic recombination of horizontally-acquired DNA cassettes resulting in a genetic mosaic in this region. Variation in the central proline-rich region has arisen from the accumulation of point mutations resulting in two distinct and divergent sequence types sharing only 55 % homology, while variation at the 3'-end of the gene is due to deletion or duplication of defined repeat units. Potential antigenic and functional variability in SfbI implies significant selective pressure *in vivo* with direct implications for the pathogenesis of *Streptococcus pyogenes*.

Small streptococci highly magnified: Dr.Manfred Rohde and Prof. Singh Chhatwal analyse the crosstalk between streptococci and human host cells using a scanning electron microscope.

Photo: Bierstedt

01.2 Virulence Factors of *Streptococcus pneumoniae*

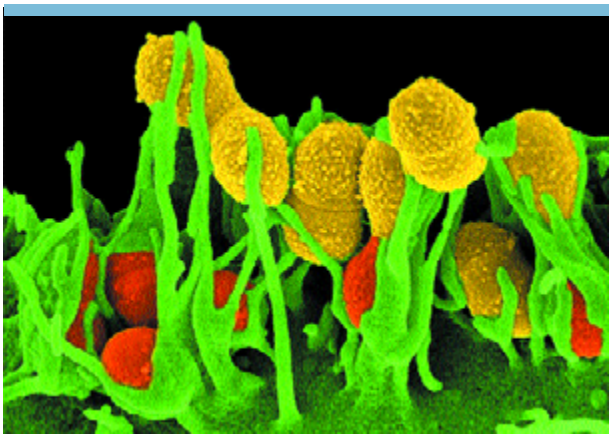
PROJECT LEADER | **Priv.-Doz. Dr. Sven Hammerschmidt*** | **Department of Microbial Pathogenicity and Vaccine Research**

PROJECT MEMBERS | Dr. Simone Bergmann | Dagmar Bracht | Christine Elm

The strategies used by *Streptococcus pneumoniae* to adhere to and invade eukaryotic cells are not well understood. The pneumcoccal research group has contributed to our understanding of pathogen-host interactions through the identification of pneumococcal adhesins. These adhesins represent targets for secretory components – the poly immunoglobulin receptor hpIgR , lactoferrin, plasmin and plasminogen as well as fibronectin. Typically, Gram-positive bacteria use the interaction with extracellular matrix proteins to adhere to and invade epithelial and endothelial cells.

The way into the cell *S. pneumoniae* binds to the polymeric immunoglobulin receptor pIgR which is produced by mucosal epithelial cells via the bacterial adhesin SpsA. A hexapeptide motif in SpsA was identified as the minimal binding motif required for binding specifically to pIgR. Further investigations showed that the hexapeptide motif in SpsA is crucial for the interaction of pneumococci and pIgR-expressing cells.

Another strategy used by bacterial pathogens to cross the mucosal barrier, as well as the blood-brain-barrier, is the acquisition of host proteolytic activity. *S. pneumoniae* binds human plasminogen and its subsequent activation by host serine protease plasmin promotes migration of pneumococci through reconstituted basement membranes.
The surface displayed alpha-enolase – designated Eno – was identified as the binding site for plasmin and plasminogen. Binding assays revealed that besides the C-terminal lysyl residues, an additional internal binding motif is crucial for the binding of plasminogen via the kringle 1-3 LBS 1 of plasminogen. Analysis of spot synthezised synthetic peptides, representing Eno sequences, identified an internal peptide of nine amino acids as the minimal second binding epitope, mediating binding of plasminogen to Eno. *In vitro* and *in vivo* experiments confirmed the role of this motif in the virulence of streptococci.



Adhesion and invasion of streptococci in human mucosa epithelial cells. The attached streptococci are coloured in yellow. In red: streptococci that begin to invade the cell. The adhesion and invasion of streptococci play an important role in the infection process.

Photo: Rohde, GBF

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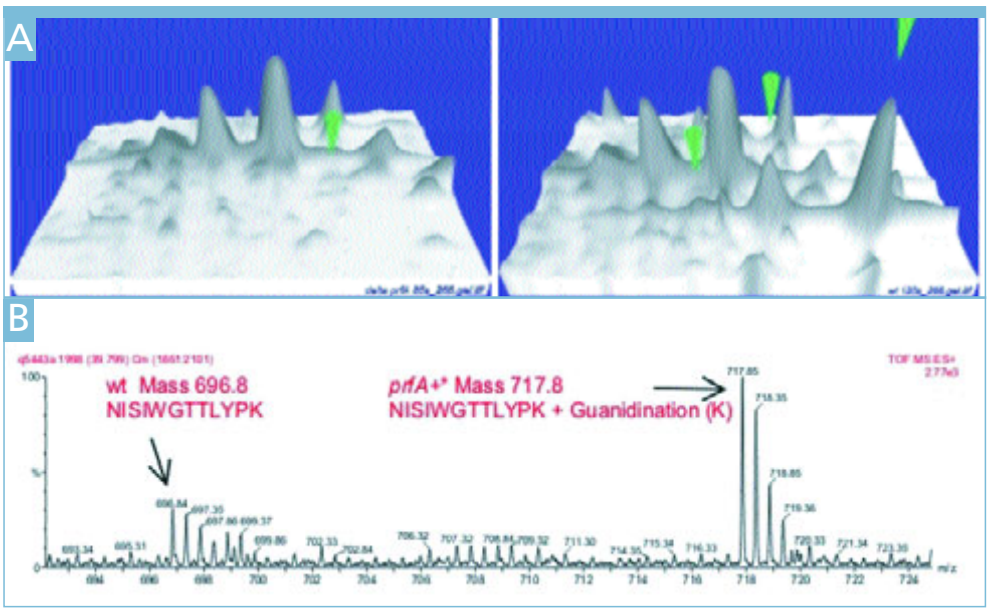
01.3 Identification and Characterisation of Bacterial Virulence Factors

PROJECT LEADER | **Prof. Dr. Jürgen Wehland** | **Department of Cell Biology**

PROJECT MEMBERS | Maja Baumgärtner | Dr. Sabine Cornelsen | Dr. Oliver Diekmann | Dr. Lothar Jänsch Dr. Uwe Kärst | Jessica Schaumburg | Kathrin Thedieck | Matthias Trost | Dr. Dirk Wehmhöhner

The Gram-positive, facultative intracellular pathogen *Listeria monocytogenes* can cause food-borne infections like meningo-encephalitits and meningitis, especially in immunocompromised persons. Part of the department is thus focusing its attention on *Listeria* virulence factors. The objective of this project is to analyse the protein patterns of *Listeria monocytogenes* using high resolution 2D-gel electrophoresis and rapid protein identification by mass spectrometry based on the completed genome sequence.

New virulence factors Since the interactions of pathogens with host cells are mediated through their external, *i.e.* secreted, cell wall-associated and membrane proteins, our investigations focused on these subproteomes. Methods for isolating and analysing defined subproteome fractions were developed and mutants analysed to facilitate identification and characterisation of *L. monocytogenes* virulence factors. This includes protein complexes and the search for potential receptor proteins on different host cells that participate in host cell – pathogen interactions. Methods of serial protein extraction using salts and endolytic cell wall digestion with protoplast formation for the isolation of secreted and cell wall-bound proteins were developed. As part of these activities, a gel-less method for comparative MS analysis suitable for rare proteins was developed and will be extended to permit quantitative analyses. Nearly 300 Listerial proteins have been identified in the course of these investigations, including the virulence factors that are already known, as well as several proteins of unknown function, mainly classified as being associated with the cell wall, transport systems and the cell surface.



- *Detection of known and putative virulence factors of* L. monocytogenes. *A: Three dimensional picture of a comparative 2D-PAGE (excerpt). Detection of a putative virulence factor, controlled by the main regulator for virulence genes PrfP (left: prfA- deletion stem, right: wild type)*
  B: mass spectrometrical quantification of a peptide of the known virulence factor Listeriolysin. Labelling of a sample of the constitutive PrfA expressing strain after guanidification of lysin residues (MCAT).

01.4 Genomes and Proteomes of Streptococci

PROJECT LEADER | **Dr. Dorothea Zähner** | **Department of Microbial Pathogenicity and Vaccine Research**

PROJECT MEMBER | Inka Sastalla

The diseases caused in humans by streptococci are very diverse. The main reason for this is the genetic differences between the particular strains involved. This project is concerned with the use of genomic methods in characterising streptococcal isolates and their association with different streptococcal diseases. It includes the development of microarrays and the creation of deletion mutants. Together with our collaborators within the "Kompetenznetzwerk Pathogenomik" – a whole genome microarray of streptococci has been developed. The genome sequences used to deduce the oligonucleotide sequences originated from strain SF370, isolated from a patient suffering from invasive disease, and strain MGAS8323 from a patient with streptococcal toxic shock syndrome.

Application of modern molecular biological tools for the identification of new bacterial virulence factors.

Photo: Bierstedt

Discovery of new virulence genes Currently, 80 % of the sequence is available. 2,150 oligonucleotides, representing all of the identified genes, have been deposited on a microarray. An additional array with a set of 50 oligonucleotides that represent known or putative virulence genes, has also been developed. This array will be used for quickly determining the presence or absence of virulence genes in a large number of streptococcal isolates from different locations, and isolated from patients with different streptococcal diseases. The isolation method for high quality RNA has been optimised and initial hybridisation trials with the "virulence gene array" and the "whole genome array" are in progress.

Additionally, several mutants with deficiencies in regulatory genes have been constructed. The characterisation of their growth behaviour, ability to adhere and invade eucaryotic cells, and the assessment of their pathogenic potential in comparison to the wildtype in the mouse model are under way. RNA from interesting candidates will be further processed for expression profiling with the aim to identify new virulence factors.

01.5 Analysis of Bacterial Drug Producers

PROJECT LEADER | **Dr. Rolf Müller | Research Group Molecular Biology of Myxobacteria**

PROJECT MEMBERS | Dr. Stefan Beyer | Dr. Ursula Bilitewski | Dr. Helmut Blöcker | Bettina Frank | Nikolaos Gaitatzis | Dr. Klaus Gerth | Dr. Frank Gross | Julia Hovermann | Dr. Herbert Irschik | Dr. Rolf Jansen Dr. Carsten Kegler | Maren Kopp | Dr. Brigitte Kunze | Inga Müller | Olena Perlova | Dr. Silke Pradella | Dr. Shwan Rachid | Axel Sandmann | Dr. Florenz Sasse | Heinrich Steinmetz | Stefan Weinig | Silke Wenzel

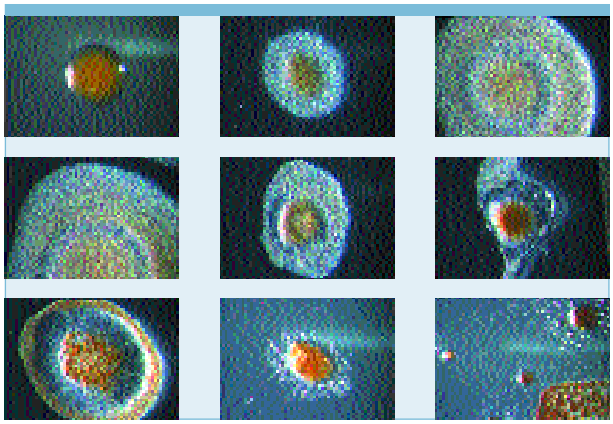
Natural products with biological activity are a very valuable source of pharmaceuticals and agrochemicals. A detailed analysis of the biology of microbial drug producers will lead to the identification of new active substances and offer additional therapeutic strategies for the treatment of infectious or metabolic diseases and cancer. In the long term, the project is aimed at finding improved methods to harness the biosynthetic potential of bacterial drug producers.

Unusual hybrid forms… The project has focused on functional analysis of the genome of *Sorangium cellulosum* So ce56, a member of the Sorangium-group which has gained a lot of interest as a producer of natural products. In the course of this project, the genome of *S. cellulosum* has been sequenced at the genome sequencing department with a 4-fold coverage resulting in less then 800 contigs of the 12,4 Mbp chromosome. A corresponding BAC-library, representing an 11-fold genome coverage, was created and spotted onto high density colony filters. End sequences of more than 100 BACs were generated.

Part of a gene cluster involved in chivosazol formation was identified by gene inactivation, which was made possible by the development of a genetic system for gene transfer and inactivation based on tri- and biparental mating. A transposon mutagenesis vector harbouring oriT, the hygromycin resistance gene, and the mariner transposon was created and can be used for mutagenesis of *S. cellulosum*. The modular and macromolecular polyketide synthases and nonribosomal peptide synthetases, especially in their unusual hybrid forms, were studied in detail, because of their immense importance for combinatorial biosynthesis.

…and new biosynthetic pathways The genes governing tubulysin biosynthesis in *Angiococcus disciformis* and melithiazol biosynthesis in *Melittangium gephyra* were cloned, sequenced, analysed and patented. Using transposon mutagenesis, overproducers of myxothiazol and tubulysin were generated and the affected genes analysed. In the melithiazol gene cluster, a novel type of S-adenosylmethiomine dependent methyl transferase involved in methyl-ester formation was found and functionally expressed. The stigmatellin gene cluster was described and used to create novel stigmatellin derivatives with biological activity. In *Stigmatella aurantiaca*, a completely unknown and primary biosynthetic pathway leading to the formation of branched chain carboxylic acids as precursors for fatty acid and secondary metabolite formation was found and biochemically described. It represents a novel branch of the mevalonate pathway and is currently under investigation at the molecular level.

A comprehensive study of steroid biosynthesis in myxobacteria was performed which resulted in identification of the first known bacterial steroid biosynthesis gene. Inhibition studies showed that the corresponding bacterial biosynthetic proteins behaved differently to eucaryotic proteins, which is important for the development and analysis of resistance mechanisms, especially against antifungal therapeutics.



Formation of fruit bodies of S. cellulosum So ce56

Photo: Gerth, GBF

01.6 Structural Analysis of Virulence Factors

PROJECT LEADER | **Prof. Dr. Dirk Heinz | Department of Structural Biology**

PROJECT MEMBERS | Stefanie Ehinger | Susanne Frese | Dr. Hans-Jürgen Hecht | Dr. Joop van den Heuvel | Dr. Birgit Hofmann | Dr. Dirk Krumme | Marina Lindemann | Dr. Hartmut Niemann | Dr. Wolf-Dieter Schubert | Dr. Victor Wray

Bacteria and other pathogens interact with their environment via individual molecules. To sense and respond to their surroundings, they recognize and bind to certain receptors in their immediate vicinity, inducing directed responses or signaling cascades. In this project, we are particularly interested in cases of molecular recognition between microbial pathogens and their human hosts – at atomic resolution. Such detailed information will aid in combating infections caused by pathogenic bacteria, viruses or parasites.

Adhesion and invasion by von *Listeria monocytogenes* The bacterium *L. monocytogenes* is the causative agent of listeriosis, a disease with a high mortality rate mainly in immuno-compromized individuals. Picked up by food, the bacterium binds to cells lining the human intestine, inducing its own uptake into these cells. Once inside a host cell, it multiplies and spreads to neighboring cells. To specifically recognize intestinal cells, *L. monocytogenes* presents a dedicated molecule on its cell surface, known as internalin A (InlA), which recognizes the human cell surface protein E-cadherin, expressed by cells of the intestinal epithelium. In the host, E-cadherin links neighbouring cells of the epithelium through its N-terminal domain which dimerizes with equivalent domains.

The crystal structure of an α-enolase dimer from Streptococcus pneumoniae.

Recently, our research group solved the structure of InlA' – the functional part of InlA – both on its own and in complex with hEC1, the N-terminal domain of E-cadherin.

Two details are of particular importance: the hydrophobic amino acid, Pro16, of E-cadherin is intimately recognized by a hydrophobic pocket on the surface of InlA. In murine E-cadherin, this proline is replaced by glutamate – making mice resistant to orally administered *Listeria*. Furthermore, a calcium ion, located between negatively charged side chains of both proteins, bridges both molecules, leading to further stabilization of the interaction. Ca^{2+}-concentration in the intestinal lumen is about a thousand-fold higher than within the cytoplasm, thus favoring bacterial invasion into the cells. Once inside the intestinal cell –surrounded by a vacuolar membrane – the lower Ca^{2+}-concentration destabilizes the complex, thus releasing the bacterium into the cytoplasm.

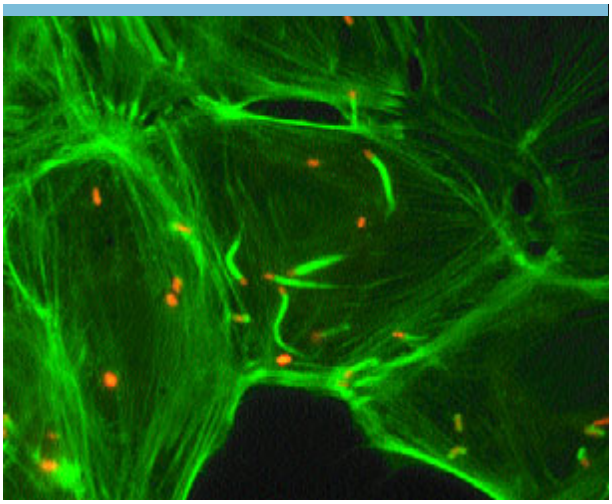
In addition to InlA, *L. monocytogenes* utilizes InlB, a second, related protein to induce its uptake into cells of other human tissues. A conspicuous arrangement of five aromatic residues presented on the concave surface of InlB' is immediately obvious, suggesting a potential interaction with the InlB-receptor cMet. By replacing these aromatic amino acids with smaller and polar amino acids, it could be demonstrated that they are essential in mediating binding to the dimeric cMet and for inducing bacterial uptake into eukaryotic cells.

Enolase from *Streptococcus pneumoniae* α-Enolase of *S. pneumoniae* is a intracellular glycolytic enzyme, which also acts as a virulence factor outside the cell, where it activates human plasminogen, leading to invasion of the bacteria of non-phagocytic host cells. The crystal structure of α-enolase shows regions at its surface that mediate the interaction with plasminogen. To further characterize the interaction between both proteins and thereby cast new light onto the mechanism of invasion, structural analysis of the protein complex is planned.

Topic 02 – Pathogenesis

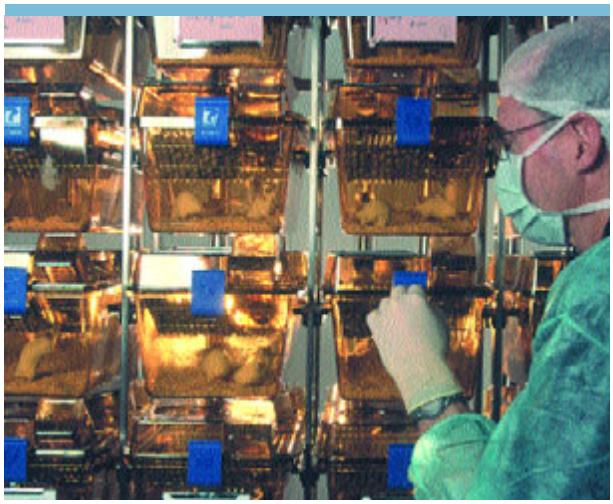
TOPIC SPEAKER | **Prof. Dr. Jürgen Wehland** | **Department of Cell Biology**

- A prerequisite for the development of new diagnostic and therapeutic strategies is a detailed knowledge of the particular infection process and of how the disease progresses. The projects comprising this topic aim at analysing and elucidating the mechanisms of pathogenicity, not only in respect to the pathogen, but also in respect to the host system, focussing on host-pathogen interactions, especially the adhesion and invasion mechanisms of Streptococci, *Listeria* and pathogenic *E. coli*. Equally important are the reactions of the host during infection with respect to its immune defence system.
  Here, pathogens have developed sophisticated virulence mechanisms which enable them to circumvent host defence systems and to survive long enough in the host for establishing an infection. In addition, the establishment of animal infection models is essential for the analysis of pathogenicity mechanisms.



- *Cells infected by* L. monocytogenes

Photo: Rohde, GBF



- *Dr. David Monner during the daily control of the mouse cages*

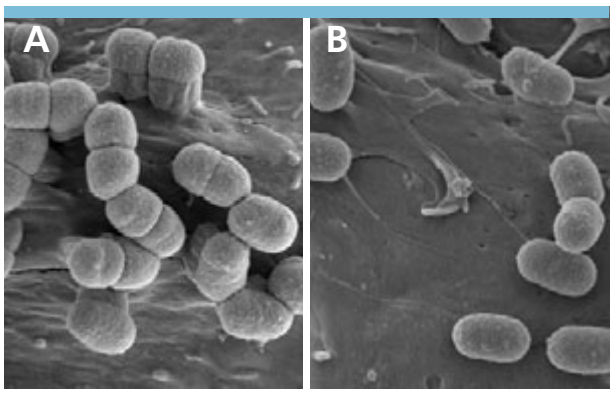
Photo: Bierstedt

02.1 Molecular Mechanisms of Pathogen/Host-Cell Interactions

PROJECT LEADER | **Prof. Dr. Jürgen Wehland | Department of Cell Biology**

PROJECT MEMBERS | Stefanie Benesch | Dr. Silvia Lommel | Anke Mateus | Dr. Sascha Pust | Dr. Klemens Rottner | Dr. Antonio Sechi

In this project, our group had focussed on the establishment and characterisation of cell lines derived from gene targeted mice lacking functional cytoskeletal components. Such cell lines are instrumental for analysing host cell-pathogen interactions at the molecular level. In particular, we analysed in detail the role of the Ena/VASP family member VASP (Vasodilator Stimulated Phosphoprotein) in intracellular *Listeria* motility, and the role of the WASP/Scar family member N-WASP (Neuronal Wiskott Aldrich Syndrome Protein) in *Shigella* motility. Our data suggest that Ena/VASP proteins, although not essential, contribute to *Listeria* motility by regulating both the formation and elongation of actin filaments at the bacterial surface.



Scanning electron microscopical analysis of the interaction of enteropathogenic E. coli *with (A) normal and (B) N-WASP-defective fibroblasts. These bacterial pathogens exploit the actin cytoskeleton in order to induce on the host cell surface pseudopode-like structures. Bacteria are residing at the tips of these pedestals (A). The bacteria still adhere to N-WASP defective cells (B) but are unable to induce pseudopodia.*

Photo: Rohde, GBF

In contrast, N-WASP was confirmed to be absolutely essential for the recruitment and activation of the Arp2/3-complex to the *Shigella* surface, and hence for its intracellular actin-based motility.

Having originally focussed on *L. monocytogenes* and *Shigella flexneri*, our studies now include enteropathogenic and enterohemorrhagic *E. coli* (EPEC and EHEC).

Actin tails Similar to *Listeria* and *Shigella*, the facultative intracellular bacterium *Burkholderia pseudomallei* induces actin rearrangements within infected host cells leading to the formation of actin tails and membrane protrusions. To investigate the underlying mechanism, we analysed the contribution of various cytoskeletal proteins to *B. pseudomallei* – induced actin tail assembly in detail. The recruitment of these cytoskeletal components to the surface of *B. pseudomallei* and into the corresponding actin tails was studied. Our results suggest that *B. pseudomallei* induces actin polymerisation through a mechanism that differs from those evolved by other intracellular pathogens that exploit the actin cytoskeleton, such as *Listeria*, *Shigella*, *Rickettsia* or vaccinia virus.

02.2 Molecular Mechanisms of Streptococcus/ Host Cell Interactions

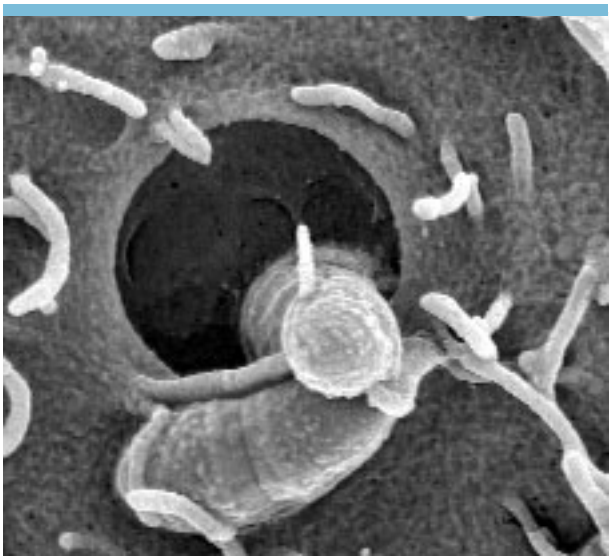
PROJECT LEADER | **Dr. Susanne Talay | Department of Microbial Pathogenicity and Vaccine Research**

PROJECT MEMBERS | Katrin Dinkla | Dr. Wouter Jansen | Dr. Manfred Rohde

The ability to colonize a host and evade its immune defence mechanisms are fundamental to infection by group A streptococci. In this project, a new colonization mechanism of streptococci was elucidated at the molecular level. Streptococci can recruit and aggregate human collagen through surface bound fibronectin – a complex recruitment mechanism triggered by a single protein. The research group demonstrated that the biological consequence of this interaction is the formation of large bacterial aggregates, preventing phagocytosis of the bacteria. Furthermore, fibronectin-mediated recruitment of collagen mediates adherence of bacteria on collagen fibers.

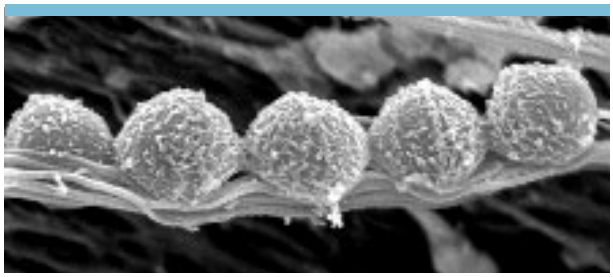
Magic hood of collagen Another highlight of this project was the identification of capsule and M3 protein as receptors for type IV collagen. This capability of M3 protein is unique. Together with the capsule, it plays an important role in direct adherence of the bacteria to collagen fibers and ultimately in colonization. Serotype M3 *S. pyogenes* isolates, as well as highly encapsulated strains, are able to bind to and colonize collagen. A serious consequence is the generation of a collagen-specific auto-immune response in the organism that may lead to destruction of collagen and tissue damage.

In addition, the cellular processes triggering reorganization of the host cell membrane and leading to intracellular uptake of streptococci were identified. Caveolae are the cellular compartments which govern the uptake process. SfbI protein is the essential bacterial factor for caveolae recruitment on the cell surface, triggering fusion to caveosomes. Streptococci located inside the cell can evade the immune system and the detrimental effects of antibiotics, thereby leading to persistence of streptococci in the host organism.



Cerveolae mediated invasion of S. pyogenes *in host cell*

Photo: Rohde, GBF



Colonization of Streptococcus pyogenes *on collagen fibers*

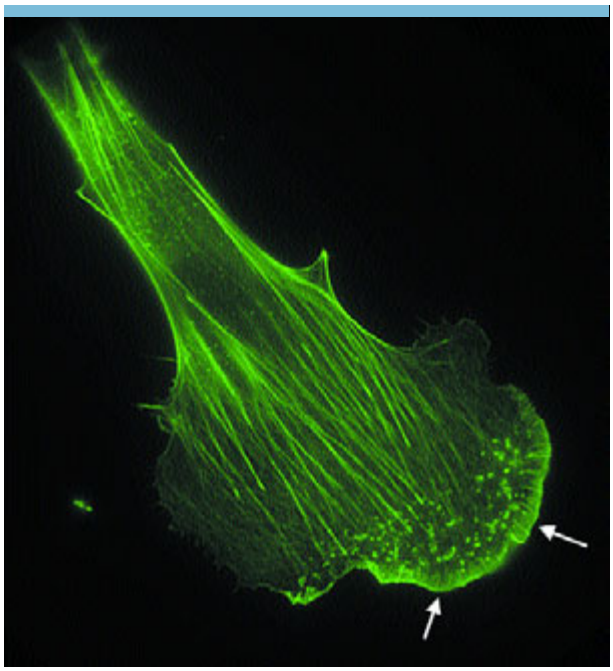
Photo: Rohde, GBF

02.3 Signalling to Actin Dynamics

PROJECT LEADER | **Prof. Dr. Jürgen Wehland** | **Department of Cell Biology**

PROJECT MEMBERS | Dr. Christian Erck | Andrea Jenzora | Anika Steffen | Dr. Theresia Stradal

The projection of cellular protrusions, such as lamellipodia and filopodia, driven by actin polymerisation at the plasma membrane is essential for cell motility and for other cellular processes. These processes are also exploited by bacterial pathogens in order to invade their host cells. This project is aimed at unravelling the molecular mechanisms underlying these events.



Fluorescence microscopical image of a motile fibroblast, revealing typical lamellipodia at the front end (arrows). The actin cytoskeleton is visualized by a specific immuno-staining.

Photo: GBF

N-WASP It has often been proposed that N-WASP plays a crucial role in the protrusion of lamellipodia and filopodia. However, our research has shown by the use of N-WASP defective cells that this protein is not essential for the formation of the above mentioned structures, but rather, is necessary for actin assembly at the surface of endomembranes associated with so-called vesicle rocketing. Our findings reveal the first distinct cellular phenotype for loss of N-WASP function and indicate that the proposed link between actin and membrane dynamics may be reflected in actin-based vesicle movement.

A new protein: PREL1 The surface protein ActA of the bacterial pathogen *Listeria monocytogenes* is responsible for actin-based intracellular motility of this pathogen. ActA recruits the Arp2/3 complex and Ena/VASP proteins from the host cell cytoplasm to support actin tail formation.

Ena/VASP proteins are important modulatory components in the regulation of cell migration. Identification of the mode of interaction between bacterial ActA and Ena/VASP proteins was an essential contribution to our understanding of how Ena/VASP proteins are recruited in the cell.

A HeLa expression library was screened with a monoclonal antibody generated to recognize the proline-rich Ena/VASP-binding consensus sequence. We identified a novel protein, which we termed PREL1 (Proline Rich EVH1 Ligand). PREL1 shares homology with the Grb7/10/14-family of signalling adaptors and has a molecular weight of 73 kDa. It could be shown that PREL1 is located mainly on lamellipodia tips and directly interacts with Ena/VASP proteins, suggesting a critical role for PREL1 in the regulation of actin dynamics.

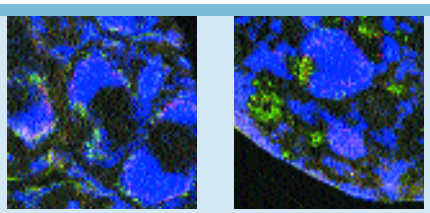
02.4 Host Reactions after Infection with Intracellular Bacteria

PROJECT LEADER | **Dr. Siegfried Weiß | Research Group Molecular Immunology**

PROJECT MEMBERS | Dr. Jan Buer | Dr. Kurt Dittmar | Nelson Gekara | Jadwiga Jablonska | Dr. Jörg Lauber | Dr. Stefan Lienenklaus | Christofer Samuelsson

On being invaded by an infectious agent, the host immediately activates its defence mechanisms by reacting against molecules in respect to which host and the microorganism are clearly distinct, like particular glycolipids or unmethylated DNA motifs. Pathogens, on the other hand, have developed virulence mechanisms that circumvent the host's defence systems, at least to a certain degree, in order to survive long enough in the host to establish an infection. Such virulence factors often also activate particular host responses. Thus, it is obvious that highly complex interactions between pathogen and host take place, which finally end in the clearance of the pathogen or the succumbing of the host to the infection.

Especially early interactions are often decisive under such circumstances. Therefore, this project studies early events that take place during an infection by the intracellular bacterium *Listeria monocytogenes*. This infection system has been extensively studied *in vitro*. In addition, a well defined murine infection model exists. Finally, the molecular genetics of these bacteria is well established, the sequence of their genome is known und many mutants are available that lack particular virulence factors.



Restructuring of the architecture of the spleen after infection with Listeria monocytogenes. *In the spleen, after intravenous infection,* L. monocytogenes *are taken up by ERTR-9 macrophages (green) that reside in the outer rim of the marginal zone surrounding lymphoid follicles. Such infected macrophages produce particular chemokines upon infection by* L. monocytogenes *and form the condensation nuclei for clusters consisting of macrophages and dendritic cells observed 24 hrs after infection. In contrast, MOMA-1 macrophages (red) that form the inner rim of the marginal zone and which are not infected by the bacteria migrate into the B-cell area indicated by the marker B220 (blue).*

Photo: GBF

Complex chemokine expression patterns Following intravenous infection, macrophages of the marginal zone of the spleen are the first target cells for *L. monocytogenes*. Since it is not yet possible to isolate sufficient numbers of such cells for a comprehensive analysis of regulated genes, we first infected an established murine macrophage cell line with an optimal number of *Listeria* and analyzed them by micro expression arrays. Most of the induced genes coded for cytokines, including chemokines. Chemokines were of special interest since these molecules are involved in attracting other cells to the place of infection and can also act as activators for other cells. The differentially expressed genes were first confirmed by Real-Time-RT-PCR using different types of macrophages that were infected *in vitro*.

Subsequently, the cytokine/chemokine pattern produced by macrophages after infection was established by Real-Time-RT-PCR *in vivo*, whereby large deviations from the pattern established *in vitro* were observed. Macrophages did not produce β-interferon, although strong expression of this cytokine was observed in the spleen. Histological studies showed that infected macrophages were associated with plasmacytoid dendritic cells. These cells are known to be the major producers of α- and β-interferons.

Detailed analysis of chemokine expression by infected macrophages *in vivo* revealed additional differences to *in vitro* expression. Some chemokines are produced at a very high rate and in remarkable quanities for a brief period, but become almost undetectable after a short time – whereas other chemokines dominate the host response. This expression pattern of chemokines, together with additional inflammatory cytokines, results in marked restructuring of the spleen's architecture.

These studies have been performed so far in BALB/c mice. Now, we have extended these experiments to two additional strains: C57Bl/6, which is more resistant to *Listeria*, and DBA/2, which is more susceptible. Deviating patterns of chemokine and interferon expression were observed in the three mouse strains tested. We hope to correlate these expression patterns with the status of susceptibility.

02.5 Pathogenesis of Streptococcus in Animal Models

PROJECT LEADER | **Dr. Eva Medina** | **Department of Microbial Pathogenicity and Vaccine Research**

PROJECT MEMBERS | Maike Bolm | Dr. Wouter Jansen | Antonia Toppel

Clinical manifestations of infection caused by group A streptococci, e. g. *Streptococcus pyogenes*, include mild diseases such as pharyngitis, but also very severe ones, like necrotizing fasciitis and streptococcal toxic shock syndrome. Several studies have suggested that host genetic factors might be involved in the predisposition of the patient to develop a mild or a severe form of streptococcal disease.

This hypothesis is supported by experimental studies of *S. pyogenes* infection in inbred strains of mice, which show marked differences in survival depending on the mouse strain. Thus, while some strains of mice (*e. g.* BALB/c, DBA/2) were very resistant and developed only a very mild form of disease after infection with *S. pyogenes*, other strains (*e. g.* C3H/HeN, CBA/J) were much more susceptible and developed very severe streptococcal infections. Identification of host immune mechanisms that contribute to the severity of infection with *S. pyogenes* in mice will increase our understanding of the genetic factors that may also determine resistance and susceptibility to streptococcal infections in humans.

Our results show that resolution of infection in resistant mice was correlated with an effective control of bacterial growth and with a moderate inflammatory response. In contrast, susceptible mice failed to control bacterial growth and responded to infection with a vigorous inflammatory reaction (significant increases in serum levels of inflammatory mediators), which cause extensive tissue destruction, organ failure, and death. A mild inflammatory response, as observed in the resistant mice, is needed to control and kill the invading pathogen. In contrast, the excessive inflammatory response observed in susceptible mice is potentially autodestructive and can be fatal. In conclusion, our results seem to indicate that the susceptibility of mice to *S. pyogenes* infection is a combination of two mechanisms: impaired capacity of immune mechanisms to kill *S. pyogenes* and genetic predisposition to generating a strong inflammatory response to streptococcal products.

***S. pyogenes* survives neutrophils** Since the late 1980s, an increased incidence of severe invasive GAS in-fections have been observed worldwide. This increase has renewed the interest in understanding virulence mechanisms of this pathogen at the molecular level. Therefore, the objective of this part of our work was to gain further insights into the strategies used by *S. pyogenes* to escape host defense mechanisms and survive in the infected host.

Neutrophils have long been known to provide significant host defence against *S. pyogenes* infection and a high number of these cells can be detected at the infection foci. We have demonstrated that an additional strategy of *S. pyogenes* to circumvent the host defences is to avoid the killing mechanisms and to survive intracellularly within the neutrophils. By surviving within these phagocytic cells, *S. pyogenes* can also exploit the free trafficking privileges of these cells within the
host to systemically disseminate from a local focus of infection. Intracellular bacteria could then establish new sites of infection by eventually escaping from the short-live neutrophils. A better understanding of the biology of streptococcal infections could be of critical importance for the design of new therapeutic interventions and treatments against *S. pyogenes.*

The confocal microscope facility at the GBF. The advantage of confocal over conventional microscopy is the possibility to observe structural components within cells and tissues in three dimensions.

Photo: Bierstedt

Topic 03 – Immunobiology

TOPIC SPEAKER | **Dr. Werner Müller** | **Department of Experimental Immunology**

- The research topic, immunobiology, studies basic mechanisms of the immune system. These mechanism normally act to defend the organism against infection. However, when it malfunctions, the immune system can turn against the body it is meant to defend, causing allergies, chronic inflammation, and autoimmunity. Analysis of these mechanisms is conducted using mouse models. The targeted alteration of genetic information in the mouse facilitates cause-orientated observation of the processes involved in disease. Intracellular systems are studied, such as signal transduction and gene regulation during the immune response. Intercellular reactions, such as T-cell tolerance, mucosal immunity, or the *in vivo* analysis of cell dynamics during immunological processes, also belong to the spectrum of research covered, as do the developmental and physiological regulation of immune defence genes and the comparative sequence analysis of the murine IgH locus.

- *Perparation of samples*

Photo: Bierstedt

- *Taking blood from a mouse for the analysis of immune cells*

Photo: Bierstedt

03.1 Signal Transduction and Gene Regulation

PROJECT LEADER | **Dr. Hansjörg Hauser | Department of Gene Regulation and Differentation**

PROJECT MEMBERS | Dr. Thomas Böldicke | Thomas Frahm | Natali Froese | Dr. Gerhard Gross | Dr. Andrea Hoffmann | Dr. Mario Köster | Dr. Andrea Kröger | Ina Niedick | Andreas Winkel | Dr. Manfred Wirth

Pathogenic attacks induce numerous activities in host cells, including pathways that lead to innate immune activation. Cytokines and inflammatory signals in turn induce alterations in the target cell's expression profiles. Microorganisms, as well as cytokines, activate gene expression through signalling cascades, involving families of proteins, that are of central importance in the regulation, not only of host defence, but also for "normal" cell proliferation, differentiation and cell death. Cellular responses to many cytokines and pathogens occur through liganded receptors and NF-κB, leading to the secretion of various modulators, such as cytokines, chemokines and interferons. This leads to the activation of multiple factors via TAK1, members of the Jak-STAT pathway and of protein kinases, such as p38, JNK, IKK-beta and PKB/Akt.

We are investigating intermediates in the NF-κB and Jak-STAT pathway and the possibility of cross-talk between different signalling pathways characterized by signalling mediators, such as TAK1/SMAD, SMAD/STAT and NF-κB/TAK1. One aim is toelucidate the network of signalling mediator interactions and to analyse the biological functions in which certain signal mediators are involved.

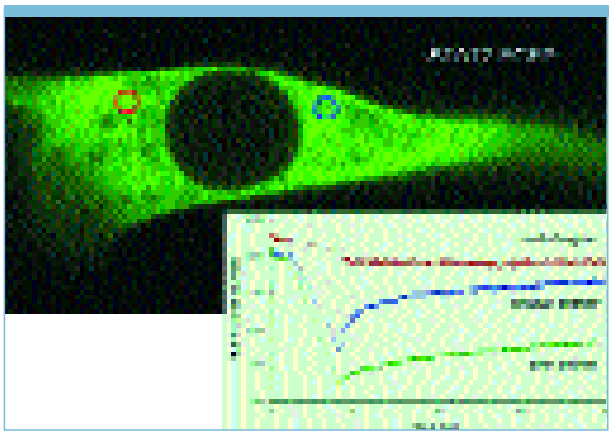
IRF-1, NRF and TAK 1 The transcriptional activator, IRF-1, which is induced by many cytokines and pathogens, reverts the transformed phenotype of oncogenically transformed cells. This is reflected by in a normalization of the cell cycle, changes in the expression of relevant proteins, as well as in phenotypic effects.

NF-κB mediates the cytokine induced transcriptional simulation of many genes. NF-κB repressing factor, NRF, is responsible for the constitutive silencing of several NF-κB promoters, including those that direct the *IL-8* and *IFN*-β genes. We have been able to show that a similar mechanism is directing the regulation of the *iNOS* gene.

TAK1 – the MAP kinase MAP3K – is a central signalling mediator activated by proinflammatory cytokines like TNF-α, IL-1 and bacterial LPS. TAK1 has the ability to interact directly with all SMAD types. The SMAD interaction with TAK1 takes place through the conserved SMAD-MH2 domain and this interaction is dependent on the presence of the active kinase domain of TAK1.

This interaction has important biological consequences. For example, BMP-dependent tissue-regeneration seems to be entirely blocked by activated TAK1 during inflammation and infection.

STAT-signalling The regulation of STAT protein activation and the kinetics of subcellular transfer during cytokine stimulation was studied. To monitor protein-protein and protein-DNA interactions during STAT signalling, diverse methods of confocal laser scanning microscopy were applied: Fluorescence Resonance Energy Transfer (FRET), Fluorescence Loss in Photobleaching (FLIP) and Fluorescence Recovery after Photobleaching (FRAP). Using the FRAP technique, the interaction of STAT2 with its interaction partner, p48, was demonstrated. Cytoplasmic anchored p48 reduced the intracellular mobility of a STAT2-GFP fusion protein compared to the effect of the interaction mutant p48¢ID.



FRAP analysis for measuring protein-protein interactions in living cells. The intracellular mobility of a STAT2-GFP fusion protein was determined by the FRAP technique in the presence of a membrane anchored wild type p48 protein or a mutant p48DID protein, respectively. The STAT2-GFP proteins in the blue circle were bleached while the red circle serves as a control region. The recovery of fluorescent molecules was measured in the control and in the bleached region over time. The plot shows examples of FRAP recovery curves. The membrane anchored wild type p48 strongly reduces the intracellular mobility of STAT2-GFP compared to the interaction-deficient mutant p48DID.

03.2 Epigenetic Principles of Gene Regulation

PROJECT LEADER | **Prof. Dr. Jürgen Bode** | **Research Group Epigenetic Regulation Mechanisms**

PROJECT MEMBERS | Dr. Alexandra Baer | Ellen Ernst | Sandra Götze | Yves Hüsemann | Martin Klar | Dr. Angela Knopp | Kristina Nehlsen | André Oumard

Whole genome sequences have become available for various eukaryotes. At the same time, there is increasing awareness that an understanding of differentiated cells requires information about higher genomic organization levels. We have shown that a particular class of the responsible DNA elements – scaffold/matrix attachment regions S/MARs – has a marked propensity for base unpairing and thereby for adopting secondary structures. Based on these findings, we have derived rules by which it is possible to define the location of functional gene domains using computer guided experiments. S/MARs have a number of attributes which make them particularly useful tools for constructing integrating and extra chromosomal transgenes with predictable gene expression systems. They increase transcriptional initiation rates using a mechanism different to that of enhancement, while at the same time alleviating the common phenomenon of locus dependent expression and suppressing silencing phenomena.

Techniques that have been developed for the elucidation of genomic organization principles will enable the rational construction of transgenic models. At suitable genomic sites it is possible to apply a set of tags. These facilitate the exchange of the initial expression cassette – usually a reporter gene – ,using recombinase mediated exchange technique (RMCE), with an analogous cassette carrying the gene of interest.

S/MAR-Database and -Performance

In cooperation with the GBF's Bioinformatics Department, we have established a S/MAR transaction database, thus providing the basis for the elucidation of common structural principles using various algorithms – tested on the human interferon gene cluster. The study demonstrates a close correlation of stress-induced duplex-destabilization SIDD minima, affinity for the nuclear matrix and biological activity in a variety of test systems. In an intergenic region, we have detected a novel type of regularly spaced S/MARs with a distinct protein recognition profile. Our hypothisis: these sites are involved in the organization of higher order chromatin folding at a level above the established periodic bent sites.

Nonviral Episomal Vector A natural S/MAR has been used to construct a novel prototype episomal vector which remains extrachromosomal in the absence of selection pressure. The major binding protein, hnRNP-U, was determined by an *in vivo* cross-linking strategy and the requirement of ongoing transcription for episomal maintenance. Antibody expression studies have been initiated using both one- and two-episome systems for co-expression of heavy and light chains.

Fragile Genomic Sites Studies have indicated that retroviral integration occurs adjacent to scaffold/matrix attachment regions in regions that otherwise have the property of fragile sites. This mechanism explains the transcriptional properties of proviruses and hints at the occurence of lymphomas which have recently been described as a consequence of gene therapeutic protocols with retroviral vectors.

Cassette Exchange System Using the RMCE principle, we were able to introduce test constructs carrying either a set of prototype insulators, a set of S/MARs or neutral DNA into various genomic sites and compare their performance. The results revealed unexpected similarities between the GC-rich insulator element HS4 and a set of AT-rich S/MARs.

Mario Köster and Sandra Götze studying the architecture of the cell nucleus with fluorescence microscopy.

Photo: Bierstedt

03.3 Posttranslational Protein Modification

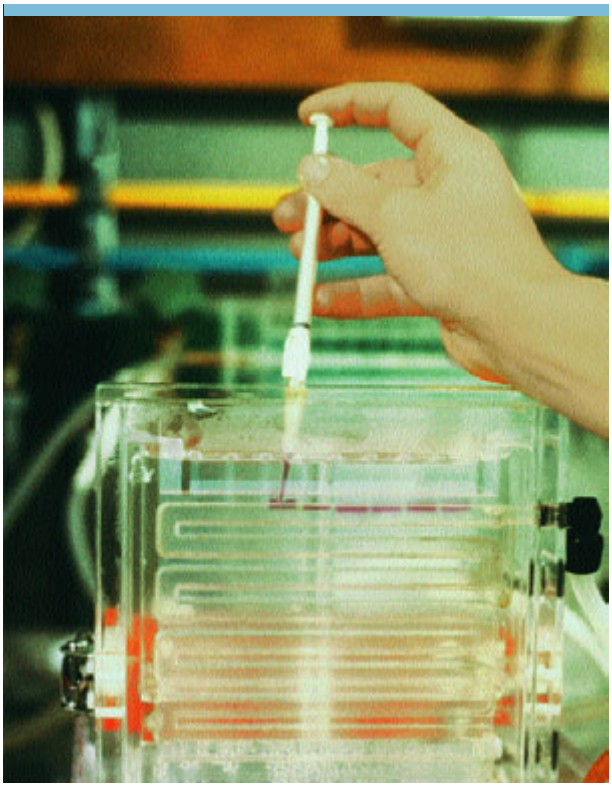
PROJECT LEADER | **Dr. Harald Conradt | Research Group Protein Glycosylation**

PROJECT MEMBER | Dr. Manfred Nimtz

The research group performed a 2D-PAGE/TOF-mapping of the proteins from human dendritic cells DCs. More than 200 protein spots have been identified – including cell surface lectins and carbohydrate receptor proteins. Selected proteins have also been analysed with respect to their posttranslational modifications, such as glycosylation, sulfation and phosphorylation.

Recombinant adenovirus vectors In collaboration with the Department Gene Regulation and Differentation, a recombinant adenovirus vector harbouring the cDNA of human EPO was prepared. After infection of a series of human and animal primary cells from various tissues, as well as cell lines, the secreted EPO was immuno-purified and analysed – including MS-MS/MS techniques – for the N-and O-linked carbohydrate chains. These cell lines – with cDNA transfection and adenovirus infection – both yielded an identical pattern of glycosylation for the EPO product. Thus, recombinant adenovirus vectors provide a versatile tool for studying the posttranslational modification repertoire of primary animal cells and cell lines.

Oligosaccharide libraries An oligosaccharide library has been established which contains more than 200 basic complex N-linked oligosaccharide structures. This library can be extended by *in vitro* glycosylation using defined glycosyltransferases to more than 1200 different oligosacharide structures. The oligosaccharide library will be used for preparing affinity matrices destined for the isolation of carbohydrate binding receptors, involved in cell-cell recognition, and for studying the biological significance of carbohydrate based receptor-ligand interactions – as a tool for approaching cell surface proteins and the enzyme machinery of the Golgi apparatus.



Preparation of a gel electrophoresis

Photo: Bierstedt

03.4 Cellular Models for Infectious Diseases

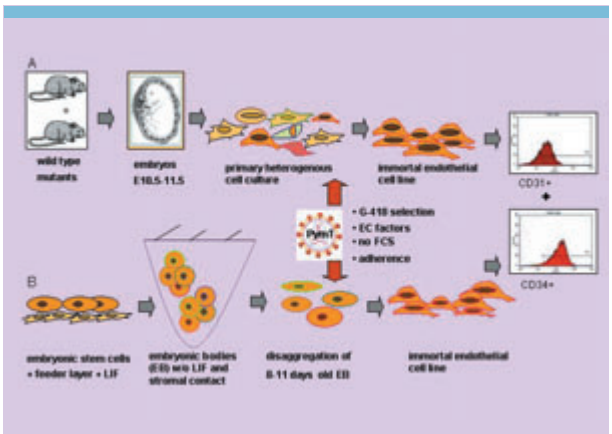
PROJECT LEADER | **Dr. Hansjörg Hauser | Department of Gene Regulation and Differentiation**

PROJECT MEMBERS | Tobias May | Dr. Peter P. Müller | Roland Schucht | Dr. Herbert Weich | Dr. Dagmar Wirth | Claas Wodarczyk

Animal studies reflect the whole complexity of an organism. However, such experiments are often extremely time consuming, difficult to reproduce and may not allow elaboration of mechanisms at the molecular detail. For these purposes, and to reduce the use of experimental animals, there is strong interest in using cell culture models to elucidate molecular details. In addition, technologies, such as gene array expression analysis, and research areas such as genomics, proteomics and systems biology, are highly dependent on homogenous, well defined and reproducible conditions. For these reasons, cell culture models are making an essential contribution to the investigation of host-pathogen interactions.

Immortalised cells Mice are preferred model organisms for infectious diseases. Whereas haematopoietic cells, such as monocytes, macrophages and dendritic cells, can often be isolated and cultured as homogenous populations, this is not generally true for cells derived from solid tissues. We are therefore establishing characterized, immune-relevant cell lines from wild-type and mutant mice or from embryonic stem cells. So far, cell lines of fibroblast and endothelial origin have been successfully established.

Whereas immortalization allows indefinite propagation of a cell line for experimental reasons, the immortalization procedure itself may influence relevant characteristics of a cell line. It has been shown that by reverting the immortalization process, certain primary cell characteristics can be restored. This is achieved by suppressing expression of the immortalization gene using a regulated promoter. First attempts at reversible immortalization have been successful. Further characterization of these cells and of various other cell types are in progress.



- *Two strategies for endothelial cell immortalisation. A: Wild type mice or mouse mutants are used to isolate mouse embryos. The isolated primary cells from the embryos are infected with a polyoma middle T (PymT) gene carrying retrovirus after explanation of the embryonic cells. PymT containing retrovirus preferentially immortalizes endothelial cells. Such endotheliomas are selected by specific culture conditions. B: Alternatively, established mouse embryonic stem cells are differentiated by changing the microenvironment. The mixture of differentiated cells is again infected with PymT virus. Resulting endotheliomas are analysed for specific mouse endothelial cell surface markers (*e.g. *CD31 and CD34).*

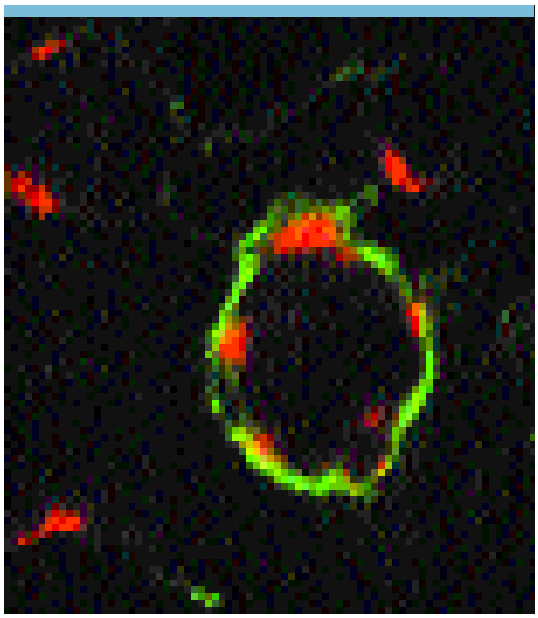
03.5 Genetic Mechanisms of Innate Immunity

PROJECT LEADER | **Dr. Andreas Lengeling** | **Research Group Infection Genetics**

PROJECT MEMBERS | Jens Böse | Laura Helming | Dr. Bastian Pasche

This project focuses on the identification of host genetic factors which play a key role in immune defence against bacterial pathogens. The objective is to use the mouse as a model system for the identification and functional characterization of infection susceptibility genes. The general idea is that infection susceptibility genes identified in mouse models can subsequently be evaluated for their possible association with genetic predisposition to infectious diseases in human patients.

Escaping the immune system Many bacterial and viral pathogens induce apoptosis in host cells at critical phases of infection. They use this mechanism to efficiently evade the host immune system. Host cells killed by programmed cell death are engulfed by macrophages within minutes of apoptosis induction. The uptake and removal of apoptotic cells by macrophages can dramatically change their inflammatory responses, in the sense that they actively suppress the secretion of pro-inflammatory mediators. The clearance of apoptotic cells is crucial in preventing post-apoptotic necrosis and the deregulation of inflammatory reactions. A protein implicated as a key regulator in the clearance of apoptotic cells is the phosphatidylserine receptor.



Expression of the phosphatidylserine receptor on peritoneal macrophages induced by thioglycollate. Immuno-staining reveals in red the phosphatidylserine receptor, in green the macrophage surface molecule F4/80.

Photo: GBF

Within the last year we have investigated the expression of this receptor in different mouse tissues and macrophage populations. We were able to show that the phosphatidylserine receptor is expressed in clusters on the cell surface of macrophages. To characterize the *in vivo* function of the phosphatidylserine receptor, different mouse models will be developed.

The vitamin D receptor – a gene for infection susceptibility? Genetic association studies in human populations link susceptibility to tuberculosis, chronic hepatitis B infections and Dengue fever to the vitamin D receptor gene *VDR*. Our hypothesis: vitamin D is an important immune regulatory hormone. To verify it, we used a mouse mutant with a defective *Vdr*-gene. It was demonstrated that *Vdr*-knockout mice are susceptible to infections with the intracellular pathogen *Listeria monocytogenes*. These experiments now provide the basis for elucidating which immune effector cells might be influenced by Vitamin D signalling.

Susceptibility to *Streptococcus pyogenes*
Infections with *Streptococcus pyogenes* can cause septic shock and multiple organ failure in humans, as well as in certain inbred strains of mice. The primary reason for the development of sepsis in the mouse infection model is a deficiency in the capability to control bacterial replication and pathogen clearance in the early phase of infection. This susceptibility is genetically determined. Using genetic linkage studies, it could be shown that genes on mouse chromosomes 2, 7 and 17 influence the outcome of infection.

03.6 T-Cell Development and Function

PROJECT LEADER | **Prof. Dr. Jan Buer** | **Research Group Mucosal Immunity**

PROJECT MEMBERS | Dr. Christian Becker | Dr. Dunja Bruder | Patricia Gatzlaff | Dr. Robert Geffers | Marcus Gereke | Ulrike Goelden | Dr. Lothar Gröbe | Dr. Wiebke Hansen | Katrin Hunger | Dr. Jörg Lauber | Dr. Andreas Matussek | Susanne Pförtner | Michael Templin | Astrid Westendorf

The research activities of this group are concerned with T-cell tolerance and mucosal immunity, focussing on the molecular biology of the interaction between the mucosal immune system and bacteria. This requires the development and use of methods for dynamic analysis of *in vivo* gene expression. Our aim is to develop, in animal models, completely new and highly effective therapies for patients with disturbed mucosal immunity, which cause inflammation and autoimmunity.

T-cell tolerance Recently, a new molecular marker for peripheral immune regulation was identified and its importance in maintaining peripheral *in vivo* tolerance demonstrated. Using a multiplex-single cell-RT-PCR, the direct expression of MHC class II molecules in island cells of the pancreas was demonstrated. Currently, this *in vivo* model is playing an important role in elucidating some basic questions relating to peripheral immune regulation.

Mucosal immunity One of the key tasks of the intestinal immune system is to develop and maintain tolerance against numerous antigens. This is reflected in the reduced ability of mucosal T-lymphocytes to be stimulated by antigens and mitogens, as well as in the production of cytokines with suppressor activities. The phenomenon of antigen-specific suppression of systemic immune-responses after application of oral antigens is referred to as the induction of oral tolerance. In this way, systemic tolerance is achieved either actively or passively. The significance of local immunological reactions in the intestinal mucosa for the pathogenesis of various intestinal diseases has become more and more apparent in the last few years. Studies with knock-out mice have shown that disrupted interactions between mucosal T cells and normal microbial flora play a special role. The molecular basis of such mucosal dysregulation and its specific treatment is not yet completely understood and still the subject of intensive research. The therapeutic manipulation of intestinal flora plays a significant role.

Mouse model system Models used to research the mucosal immune system of the intestine have become well established and already produced very promising results. The group is also developing a TCR transgenic mouse model for studying disturbances of the immune system associated with the mucus membranes of the lung. In this project, hemagglutinin –a selected antigen in the alveolar epithelium of TCR-HA mice – is being expressed and studied to see if modulation of the lung's mucosal T-cell system can be achieved with the help of recombinant bacteria expressing a defined antigen in the gastro intestinal-tract. The model system has been established and its molecular characterisation is now being carried out.

Another focus of our research is the complex interaction – so called Cross-Talk – of *E. coli* 0157 EHEC with host cells. Together with the Institute of Medical Microbiology at the MHH, the impact of EHEC toxins on endothelial cells was studied.

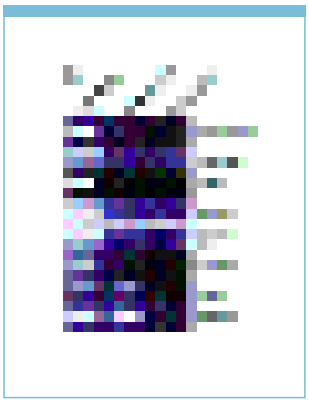
Cell sorting of T-cell lymphocytes

Photo: Bierstedt

03.7 B-Cell Subpopulations

PROJECT LEADER | **Dr. Siegfried Weiß | Research Group Molecular Immunology**

PROJECT MEMBERS | Sandra Düber | Dr. Karsten Kretschmer | Isabell Rode | Britta Störmann



- *Genes differentially expressed in isolated B1a cells from the spleen and the peritoneal cavity revealed by RT-PCR. Cells were sorted according to IgM and CD5 expression, and RNA was extracted from about 100,000 cells. PCR was performed after reverse transcription using primers specific for VCAM-1 (Vascular cell adhesion molecule 1), CD206 (mannose receptor), lipoprotein lipase, hydroxyprostaglandin dehydrogenase 15 (NAD), Spi-C (transcription factor) and CCR3 (chemokine receptor). The house keeping genes CD5 and HPRT were used as control for integrity and appropriate concentration of cDNA. Water was used as control for the specificity of the PCR reaction.*

Antibody producing B cells can be subdivided into follicular B2 cells, B1a, B1b and marginal zone B cells. Follicular B2 cells are continuously generated in the bone marrow of adult individuals. They respond specifically after introduction of antigens by differentiation into plasma cells, as well as by undergoing isotype switching and somatic hypermutation. They can also develop into memory B cells. Marginal zone B cells are closely related to follicular B2 cells. However, due to their rapid proliferation ability and their location in the spleen, they are believed to provide a first line of antibody defence against blood born infections. B1a and b cells dominate body cavities like the peritoneum, although B1a cells can also be found in spleen. Such B cells are believed to be responsible for the production of most of the serum IgM and of natural antibodies. Thus, they are also considered to provide a first line of antibody defence against infection. B1 cells are self renewing – they are normally only generated in foetal and neonatal phases of development, but not in adults.

Biology of the first line of antibody defence

In order to understand the physiology of the B-cell populations involved in the first line of antibody defence, our research group is using a recombinant mouse that expresses high levels of a lamda 2 immunoglobulin light chain as transgene. The B-cell populations found in these mice are exclusively of the first line of defence. Normal numbers of marginal zone B cells are found, B1a cells dominate the peritoneum and spleen; no follicular B2 cells can be detected in such mice.

First, we analysed the heavy chain repertoire of B cells under the restriction of the transgenic light chain. The peritoneal B1a compartment was dominated by a few heavy chains often derived from independent rearrangement events.

Such sequences were not detectable in B cells from fetal liver, the primary lymphoid organ were B1a cells are generated. Thus, strong antigen selection, most likely by an autoantigen, must be acting on B1a cells in the adult peritoneum. To define the antigen that selects such antibodies, several hybridomas from such dominating clones were established. These experiments were extended to the repertoire of B1a cells of the spleen. Little overlap was observed between the sequences derived from peritoneum and the spleen, suggesting little exchange of B1a cells between these locations. Transfer experiments confirmed this finding. However, it was shown that peritoneal B1a cells have the capacity to migrate to the spleen when transferred to mice that have no lymphocytes.

The unique situation of a mouse that only contains B cells of the first line of defence allowed us to study the genetic programme of these B-cell subsets. Sorted cells of the various subpopulations were used for analysis using micro expression arrays. To obtain sufficient amounts of RNA for hybridisation, a two fold RNA amplification step was performed before use. This analysis cast light on the physiological differences between peritoneal and splenic B1a cells with regard to T-cell interaction and activation status. These experiments were flanked by functional tests to confirm properties that were suggested by the gene expression analysis.

03.8 Biology of the Immune Defense

PROJECT LEADER | **Dr. Werner Müller | Department of Experimental Immunology**

PROJECT MEMBERS | Dr. Mariella Bollati Fogolin | Anne Fleige | Dr. Martin Hafner | Rolf Hühne | Carola Neffgen | Ida Retter | Dr. Angela Schippers | Samira Schroeder | Gudrun Wessel

The immune system is essential for defense against pathogens. It is composed of specific organ structures consisting of many different cell types that show extensive migration activities within the body. The cytokine network is one of a number of mechanisms which tightly regulate interactions between these cells. Our research group is currently analysing how lymphocyte migration is regulated during immune response, and how specific cytokines regulate the immune system during host defence.

Chronic inflammation of the bowl The complete mouse genome sequence is now known. There are two ways to specifically inactivate selected genes in the mouse germ line. One method leads to complete inactivation of the respective gene. The other, more sophisticated method, allows inducible and cell type specific inactivation of genes in the adult mouse. These animal models are particularly useful, since they mimic what happens in acquired diseases in humans. This method of targeted gene inactivation was applied to two groups of genes, to cytokine and cytokine receptor genes, as well as to homing receptor genes. The main focus of our studies was the gut associated immune system, dysregulation of which leads to severe chronic inflammation of the bowl, resulting in diseases such as *Morbus Crohn* and *Colitis ulcerosa*. The function of the gut associated immune system is not only studied in the undisturbed mouse mutant, it is also analysed in mice infected with bacteria that can cause strong inflammatory responses of the gut – in particular the consequences of *Yersinia enterocolitica* infections.

Generating mouse models The gene-targeting laboratory helps other GBF researchers to generate specific mouse mutants with genetic modifications that alter their immune system. This systematic approach to targeted mutations in mice will result in a valuable and expanding collection of mouse mutants for the research of the immune defense.
The application of bioinformatics tools complements our work with genetically modified mice. A publicly accessible sequence analysis server has been set up (http://ngfn-blast.gbf.de/), whose functions include the comparative analysis of mouse and human genes, which is essential for the development of mouse mutants. In addition, our research group is analysing a big gene cluster that is required for the generation of immunoglobulins. Immunoglobulins are effector molecules for the immune defense and are of particular importance for the elimination of pathogens from the body.

Embryonal stem cells from mice are studied under the microscope.

Photo: Bierstedt

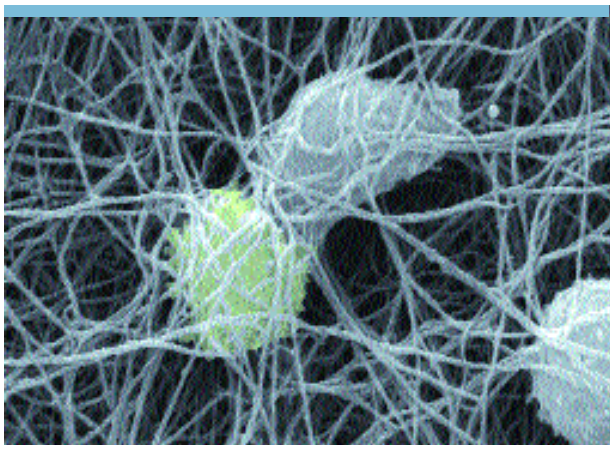
03.9 Imaging Cellular Dynamics of Immunological Processes

PROJECT LEADER | **Dr. Matthias Gunzer** | **Research Group Immunodynamics**

PROJECT MEMBERS | Anja Hillmer | Michael Templin

Immunity can broadly be divided into a humoral and a cellular arm. The humoral part is mediated by soluble factors such as antibodies or complement and the cellular part by whole cells, like T cells, B cells and dendritic cells (DC). While humoral immunity is only indirectly observable by looking at its effects, cells can be directly visualized "at work".

Migration of dendritic cells As antigen presenting cells, DC are at the beginning of every new cellular immune response. They reside in the periphery of the body, where they take up invading pathogens and transport them to draining lymph nodes to present them to T cells. Although central to cellular immunity, this transportation process has not been observed directly until now and nothing is known about its *in vivo* dynamics. There are numerous situations, where a defect in the generation of immune responses might be explained by disturbed DC migration. In the case of the immunotherapy of cancer, which now tries to make use of DC as carriers of tumour antigens, a major unsolved problem is the optimal route of DC application to the patient without disturbing their inherent migration potential. Thus, being able to visualize normal and defective DC migration *in vivo* would provide a useful tool for gaining an insight into this basic process, would help optimize protocols for vaccination programmes, and increase our understanding of disease processes.



B cells engaging an antigen specific T cell during the process of T-cell activation. The "spaghetti"-like structures represent an artificial extracellular matrix consisting of 3-D collagen fibres.

Photo: GBF

T-APC cell interaction Another aspect of cellular immunity, which is currently being studied intensively, is the physical interaction of T cells with antigen presenting cells (APC) during antigen presentation. While most of the work underlying current theories of T-APC interaction has been performed in vitro, only very recently imaging in explanted lymphatic tissue has shed light on the very dynamic migration processes going on in real lymphatic tissue. Such studies may lead to an entirely new way of thinking about of how T cell activation is achieved *in vivo* and what goes wrong in the case of disease or lethal infection.

Microscopy of living tissues Seeing is believing (and understanding). The ultimate aim of our reseach group is the visualization of cellular immunity taking place within its natural environment – a non-invasive approach using state of the art microscopy techniques. We want a comprehensive and literal insight into the biophysical dynamics underlying cellular immune processes. This technique will remain a major tool for generating and testing working hypotheses. At the same time, we are working at imaging explanted, and later *in situ*, tissues of the mouse by using time-lapse confocal and two-photon microscopy. The two-photon microscopy technique is able to generate high resolution images deep within vital tissue. To get a complete picture, images must be obtained both at sites of immune induction – in lymphnodes, spleen and gut – and at sites of immune intervention – in gut, skin and tumour-metastasis. Once the technique has been established, it is planed to use genetically engineered mice – carrying dye-tagged molecules such as MHC II or CD3 and/or defined genetic defects – as well as standardized tumour-, infection- and allergy-models to analyse the impact of ongoing disease on the cell-physiological parameters of immunity.

Topic 04 – Prevention and Therapy

TOPIC SPEAKER | **Priv.-Doz. Dr. Dr. Carlos A. Guzmán** | **Research Group Vaccine Research**

- One third of all deaths occurring each year worldwide are directly caused by infectious agents. Microorganisms are also responsible for at least 15 % of new cancers and are involved in the pathogenesis of chronic non-infectious diseases. The treatment of infected patients is rendered difficult by the emergence of multi-drug resistance. Thus, there is an urgent need to develop new tools to prevent and treat infectious diseases. The development of these tools is the main aim of this topic.

The anti-infective discovery project focuses on the identification of active compounds obtained from microbial sources. Complementary strategies based on combinatorial chemical synthesis are employed to search for small molecules with anti-infective activity. A novel steroid-like metabolite was isolated from *Sorangium cellulosum* with selective activity against mycobacteria. Epothilone B-amine, first synthesized at the GBF, is being tested in clinical trials as anticancer agent.

In the antigen delivery systems and vaccines project, tools for immune intervention are being researched and subsequently developed to create vaccines against specific diseases. Since most infectious agents have to pass through or are restricted to the mucosae, the development of mucosal vaccination procedures constitutes a priority. A synthetic derivative of the *Mycoplasma*-derived, macrophage-activating lipopeptide, MALP-2, was found to be a potent mucosal adjuvant. Novel, attenuated *Salmonella* strains were identified, which exhibited an adequate safety and immunogenicity profile as vaccine carriers.

- *Preparation of samples for gas chromatographic analysis*

Photo: Bierstedt

04.1 Synthetic Combinatorial Molecular Repertoires

PROJECT LEADER | **Dr. Ronald Frank | Research Group Molecular Recognition**

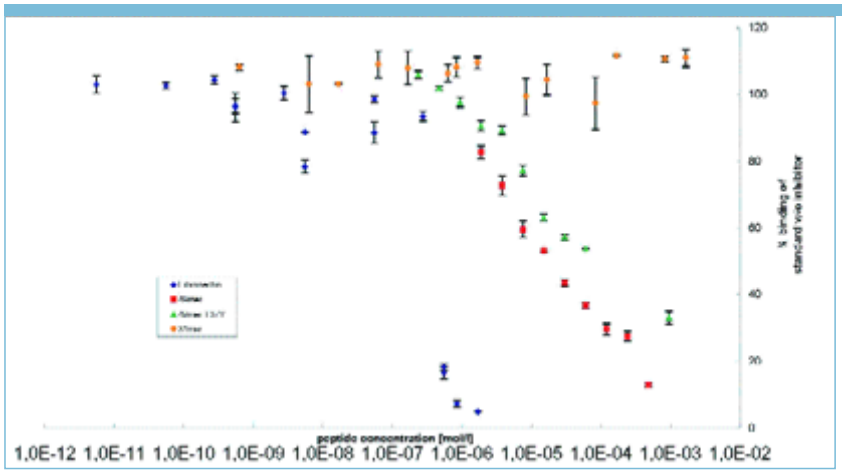
PROJECT MEMBERS | Dr. Antonius Dikmans | Undine Felgenträger | Agnes Hahn | Dr. Gerhard Höfle | Dr. Kathrin Michaelis | Dr. Michael Morr | Dr. Jutta Niggemann | Dr. Rene Rübenhagen | Dr. Werner Tegge | Dr. Norbert Zander

Complementary to the more classical approach of discovering new active substances, our group is pursuing an alternative, empirical search programme, utilizing simultaneous and parallel chemical synthesis. The combinatorial synthesis and screening technologies for peptide and small molecule libraries that have been developed will continue to be applied in the systematic analysis of protein-protein interactions and their selective inhibition. This will be further advanced and extended in the search for new compounds with antibiotic, chemotherapeutic and immunomodulatory activities.

Inhibitors of the cellular invasivity *Streptococcus pyogenes*, a member of the Group A Streptococci (GAS), is known to evade the immune system and the effects of drugs by internalization into epithelial cells. A project, started in collaboration with the GBF Department of Microbial Pathogenicity and Vaccine Research, aimed at interfering with this pathogenic mechanism. Peptides with defined sequences and peptide libraries were generated and utilized in a specially adapted microtiter plate based assay. The screen was used to investigate the binding of non-pathogenic *S. gordonii* to fibronectin, a key step in the invasion process. The high throughput assay allows parallel evaluation of hundreds or thousands of different compounds for their inhibitory potency. Short peptide candidates were identified that will serve as starting points for the develop-ment of more specific and *in vivo* applicable structures.

Synthetic MALP-2 – a promising adjuvant for nasal vaccination Most pathogenic organisms attack the human body via the mucosa. An early immune defense within this tissue would be the most effective protection. The *Mycoplasma*-derived macrophage-activating lipopeptide MALP-2, discovered at the GBF, represents a promissing adjuvant to stimulate the immune response to externally administered antigens in the mucosa. The chemical synthesis of this compound allowed a detailed structure-activity study: We successfully demonstrated a strong mucosal immune-stimulatory activity of synthetic MALP-2 in mice. The research group will now design and produce analogues of MALP-2 with optimised properties. These adjuvants will have good chances of being applied in human medicine.

The "Drug Discovery" Machine The company Evotec OAI AG is coordinating a joint project within the BMBF programme "Diagnosis and Therapy with the Help of Molecular Medicine". In the context of this project a special combinatorial synthesis programme was developed and adapted to a miniaturised high-throughput screening technology. By selective mild degradation of 14 natural products from GBF's Natural Product Collection, 34 unique new building blocks were obtained. These were incorporated into novel compounds by solid phase synthesis utilising SPOT-synthesis technology. Suitable high-through-put logistics was established and more than 55,000 compounds could be provided.



Inhibition of the binding of Streptococci to labelled fibronectin by synthetic peptides and by competitive unlabelled fibronectin. The peptide sequences are derived from the bacterial fibronectin binding protein SfbI.

04.2 Biology of Microbial Bioactive Compounds

PROJECT LEADER | **Prof. Dr. Gerhard Höfle | Department of Natural Product Biology**

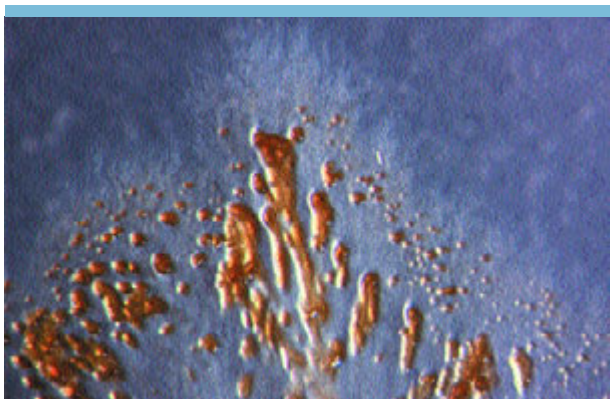
PROJECT MEMBERS | Dr. Ursula Bilitewski | Dr. Abass Yasser Elnakady | Dr. Meike Genrich | Dr. Klaus Gerth | Dr. Björn Henze | Dr. Herbert Irschik | Dr. Brigitte Kunze | Gaber Mersal | Dr. Hans Reichenbach | Dr. Florenz Sasse | Janine Wendler

The search for novel natural products with biological activity against bacterial, fungal and mammalian cells was continued, based on the existing collection of myxobacteria and around 100 newly isolated strains. An increasingly important group of compounds from these organisms is the tubulin inhibitors: epothilone, tubulysin and disorazol. Their mechanism of action as anticancer agents was further investigated in greater detail. Preclinical development was pursued in collaboration with different industrial partners, while phase II clinical trials of the semisynthetic epothilone B-lactame was successfully completed by Bristol-Myers Squibb.

New Production Organisms The isolation of myxobacteria from soil samples is well established nowadays, and strain collections of myxobacteria are available worldwide. Now, unexpectedly, unconventional isolation and cultivation conditions yielded new groups of myxobacteria with hitherto unknown physiologies. From habitats with increased salt concentration, numerous halotolerant and several halophiles, requiring 2 % sodium chloride for optimal growth, were isolated. The latter represent a new genus of myxobacteria, according to 16S r-DNA sequencing. Also, pH tolerant strains, growing at around pH 5 and 9, were found, and for the first time mesothermophiles were isolated, requiring 42 – 43° C for optimal growth. With generation times of 2 – 3 hours, these strains may be ideal hosts for the expression of biosynthesis genes.

Slag heaps from a potassium salt mine near Königslutter (Lower Saxony); non-marine salt-containing biotopes as source for halophilic bacteria.

Photo: Gerth, GBF



Swarm colony of a halophilic myxobacterium isolated from a soil sample taken at the foot of the slag heap.

Photo: Gerth, GBF

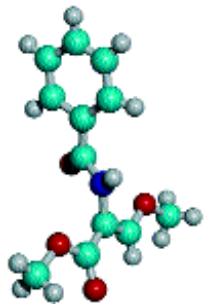
04.3 Chemistry of Microbial Bioactive Compounds

PROJECT LEADER | **Prof. Dr. Gerhard Höfle** | **Department of Natural Product Chemistry**

PROJECT MEMBERS | Dr. Nicole Glaser | Dr. Thorsten Jahn | Dr. Rolf Jansen | Dr. Usama Karama | Dr. Thomas Leibold | Dr. Jutta Niggemann | Heinrich Steinmetz | Larissa Vollbrecht | Dr. Peter Washausen

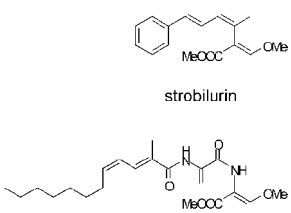
In the period covered by this report, 7 novel groups of metabolites were isolated from myxobacteria, acting predominantly on Gram-negative and Gram-positive bacteria. Preliminary structures were obtained for bysso-chloren, a complex chlorine-containing polyketide, and a steroid-like antibiotic acting selectively on mycobacteria.

Cyrmenin In screening for antifungal compounds, a *Cystobacter armeniaca*- and an *Archangium gephyra*-strain were found to produce a novel group of unsaturated N-acyl dipeptides, named cyrmenins. According to spectroscopic data and X-ray analysis of a synthetic model compound, the cyrmenins are (Z)-β-methoxy-acrylates

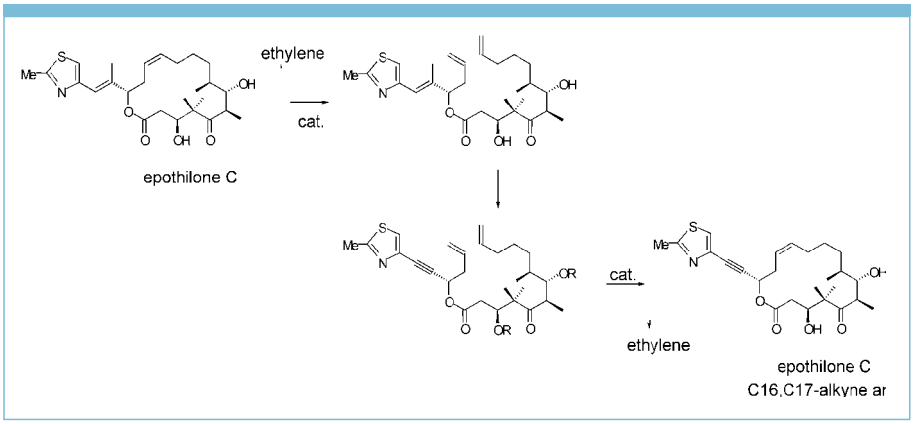


X-ray crystal structure of a synthetic cyrmenin model compound exhibiting the characteristic twisted conformation of the (Z)-β-methoxy acrylate pharmacophore required for antifungal activity.

linked in α-position by a nitrogen atom to the rest of the molecule. Thus, these compounds may be considered as aza-analogs of strobilurin, a class of compounds which has been used to develop commercially successful fungicides for plant protection in recent years. Judging by their different biosynthetic origins – polyketide versus peptide – the cyrmenins are an independent invention of myxobacteria. The antifungal activity of cyrmenin and its inhibition of electron transport at the cytochrome bc_1 complex of the respiratory chain were found to be in the same range as for strobilurin.



Semisynthesis By oxidative and hydrolytic degradation of complex natural products, a library of chiral building blocks for combinatorial synthesis was prepared. As an alternative and for the first time, olefin cross-metathesis was also applied to the degradation of olefinic natural products. To exemplify this, the C12,C13 double bond of epothilone C was cleaved in the presence of ethylene and Grubbs' metathesis catalyst. After replacement of the thiazole side-chain with a synthetic building block carrying a triple bond, the macrocycle was closed again by olefin metathesis. By this route, the C16,C17-alkyne analogs of epothilone A and C were obtained in 6 and 5 steps, respectively.



04.4 Antigen Delivery Systems and Vaccines

PROJECT LEADER | **Priv.-Doz. Dr. Dr. Carlos A. Guzmán | Research Group Vaccine Research**

PROJECT MEMBERS | Heike Bauer | Stefan Borsutzky | Dr. Dunja Bruder | Dr. Jan Buer | Dr. Thomas Ebensen | Dr. Claudia Link | Dr. Faiza Rharbaoui | Dr. Kai Schulze | Dr. Lothar H. Staendner | Dr. Siegfried Weiß

Vaccination is the most cost-effective strategy for the prophylaxis of infectious disease and is now also becoming a powerful tool in the prevention and treatment of a broader range of diseases. The main aims of this project are the development and validation of tools and strategies for the delivery of antigens or DNA vaccine constructs, as well as their subsequent development to vaccine candidates against specific diseases. The optimization of immunogenicity in antigens delivered by the mucosal route constitutes a priority, since mucosal vaccination allows us to trigger an immune response at the site where the first line of defense against infections is laid.

MALP-2 as mucosal adjuvant The potential as an adjuvant of a synthetic derivative, S-[2,3-bispalmitoyloxy-propyl] cysteinyl-GNNDESNISFKEK, of the *Mycoplasma*-derived macrophage-activating lipopeptide, MALP-2, was evaluated. The studies demonstrated that MALP-2 is a potent adjuvant when co-administered with a soluble antigen by the intranasal and parenteral routes. Up to 3500-fold enhanced antigen-specific serum IgG titers and cellular responses were observed after vaccination. The mucosal immune system was also efficiently stimulated after nasal vaccination (36 and 23 % of antigen-specific IgA in lung and vaginal lavages, respectively). Functional studies showed that there is a recruitment of antigen presenting cells with increased expression of MHC-class I and co-stimulatory molecules in the nasal associated lymphoid tissues from MALP-2-treated mice.

Stimulation of long-lasting protection against *Streptococcus pyogenes* Protective immunity against *S. pyogenes* can be induced by intranasal vaccination with the fibronectin-binding domain of the SfbI protein, the H12 fragment, co-administered with the B subunit of the cholera toxin (CTB) as mucosal adjuvant. However, intranasal administration of A-B moiety bacterial toxins or their derivatives has been associated with potentially severe side effects. Since the SfbI protein exhibits adjuvant properties, we investigated whether vaccination with the H12 fragment alone is sufficient to promote long-lasting protection. The results demonstrated that immunized mice are protected against challenge with a lethal dose of *S. pyogenes*, given 36 or 110 days after primary vaccination, to the same extent, regardless of CTB incorporation. The adjuvant properties exhibited by the fibronectin-binding domain of the SfbI protein strengthen the potential of this antigen for inclusion in multi-component vaccines against *S. pyogenes*.

Dr. Carlos Guzman is analyzing an agar plate.

Photo: Bierstedt

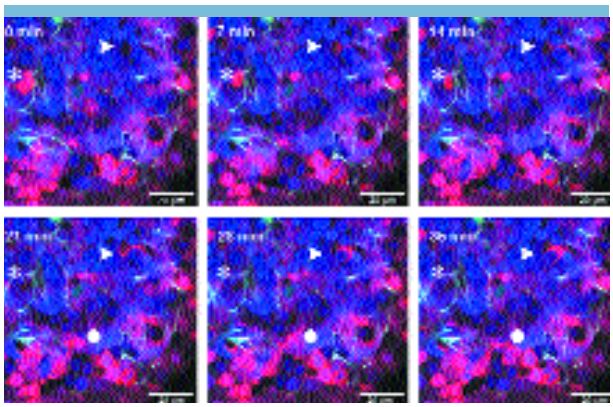
04.5 Therapeutic Cellular Vaccines

PROJECT LEADER | **Dr. Werner Lindenmaier | Department of Gene Regulation and Differentiation**

PROJECT MEMBERS | Dr. Kurt E.J. Dittmar | Dr. Andrea Kröger | Lars Macke | Carsten Wiethe

For the development of cell-based therapeutic vaccines against tumours and persistent infections, a dual strategy is followed: On the one hand, developments for clinical application are pursued, emphasizing regulatory issues and the GMP-compatible production of cells and vectors. On the other hand, cell culture and murine model systems are employed to define relevant co-stimulatory factors and cell interactions for future improvements.

Interaction between antigen presenting cells and effector cells For the functional characterization of antigen presentation, immunological assays and imaging techniques for lymphoid tissues were developed in co-operation with Dr. Manfred Rohde (Department of Microbial Pathogenicity and Vaccine Research). High-resolution confocal microscopy was used to follow cell migration and dynamic interaction *in vivo* and *in vitro* in murine lymph nodes and spleens. In cooperation with other GBF research units, the influence of infection and genetic modification on cell interactions in lymphoid organs was analysed.



Migration of living cells in murine lymph nodes
Confocal laser scanning microscopy was used to take serial images. Emigrating (*) and immigrating (➤) lymphocytes and makrophages (•) are marked. Cell nuclei, cytoplasm and reticular fibers are stained in blue, red and green, respectively.

Photo: GBF

Adenoviral modifications and cellular functions Adenoviral vectors encoding tumour associated antigens, immunomodulatory molecules and reporter genes were constructed for efficient transfer and controlled, coordinated expression of multiple genes in primary cells. In general, cellular functions other than the desired ones are not affected by adenoviral gene transfer. Infection with the replication deficient adenoviral vectors did not greatly alter cellular physiology, as shown by analysis of post-translational modification, cell surface markers and DNA array data.

From murine model to clinical applications The structural and functional properties of modified cells – especially antigen-presenting cells like dendritic cells and macrophages - and the influence of immunomodulatory genes were monitored in murine model systems and *ex vivo* with human cells. In cooperation with a research group at the SIV/Albert Sakzewdi Virus Research Centre, Brisbane, Australia, enhancement of an HPV-E7 specific immune response after vaccination with adenovirally modified DC expressing E7 and co-stimulatory molecules was demonstrated.

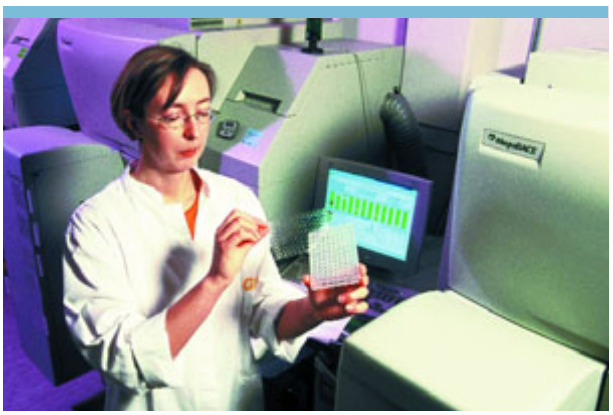
In a transplantable tumour model with inducible IRF1, activation leads to the induction of a protective, tumour-specific immune response. In order to investigate the ability of IRF-1 to induce specific immune responses in other tumour systems, an adenoviral vector expressing IRF1 was established. Tumour cells infected with IRF1 expressing adenoviruses show the same phenotype as cells stably expressing IRF1-inhibition of cell proliferation, increased MHC class I expression and IFN-β secretion.

For the production of clinical grade adenoviral vectors by the GBF S2/GMP unit, standard operating procedures and validation data were acquired. Prerequisites for the production of genetically modified dendritic cell for vaccination were established. In cooperation with industrial and clinical partners, standard protocols for the preparation of dendritic cells from leukapheresis samples were developed.

Programme “Comparative Genome Research”

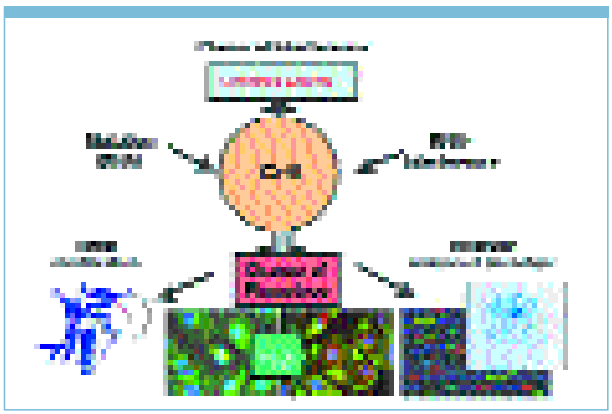
PROGRAMME SPEAKER | **Dr. Helmut Blöcker** | **Department of Genome Analysis**

- Pathogenesis depends on genotype and phenotype - conditions, including inherited genetic defects or dispositions and factors such as age, lifestyle, host-pathogen interactions and environmental stress. The comparative analysis of genome information is an essential element in studying genotype - phenotype relationships for both prognostic and diagnostic aspects in health care. In addition, the role of individual genes within the cell and their interactions in cell complexes and networks (*i.e.* tissues), as well as their translational and post-translational regulation, still remain to be elucidated. Comparative genome research can combine model-driven experimental approaches with information-driven computational and theory-based data interpretation. Thus, this research programme combines the experimental functional characterization of genomes with comprehensive genome-based bioinformatics.



- *Carola Berg prepares 96 samples for the simultaneous analysis of their DNA sequence in a capillary sequencer.*

Photo: Bierstedt



- *Three complementary systematic approaches for the functional genome analysis.*

01 Generation and Exploitation of Genomic and cDNA Sequence Data

PROJECT LEADER | **Dr. Helmut Blöcker | Department of Genome Analysis**

PROJECT MEMBERS | Dr. Michael Böcher | Frank Gößling | Michael Jarek | Bernard Neelen | Dr. Gabriele Nordsiek | Rosalila Peneido | Maren Scharfe | Dr. Oliver Schön | Harold Stiege | Dr. Maoyuang Yang

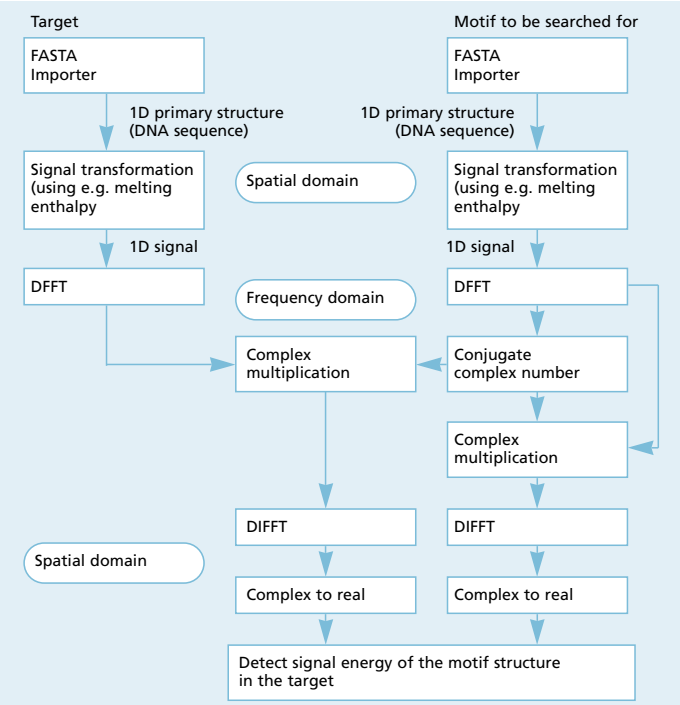
The goal of this project is to carry out genotype-driven genome research and to support phenotype-driven genome research. The former usually starts off with global analyses of long stretches of genomic DNA, or even of entire genomes, involves the application of various bioinformatics tools, and finally results in laboratory experiments used to test or develop hypotheses. All three stages – sequence analysis, bioinformatics and laboratory experiments – have been realised, either in the frame of this project or in collaboration with other projects.

Sequence analysis projects The GBF houses an active genome laboratory which covers all necessary steps of sequence analysis, from the generation of BAC libraries down to in-depth analysis using bioinformatics, and has been involved in a number of successful international collaborations. Currently, up to about 6,000 clones can be analysed per day. Annual capacity is now well above 10 megabases, which can be studied in detail using bioinformatics. Furthermore, a number of genes are selected from those which have been newly analysed here and are submitted to gene expression experiments, either in cell-free systems or in *E. coli*. Isolated proteins are then further analysed for various functional aspects.

Following completion of the EU-projects relating to *Listeria* and *Arabidopsis thaliana*, the group is continuing to make a major contribution to the German Human Genome Project. So far, more than 8 Mb of chromosomes 21 and 9 have been sequenced and annotated. The outstanding result of this activity was the publication of the sequence of human chromosome 21 and its bioinformatical analysis, completion of the working draft phase of the human genome project and the presentation of the entire human genome sequence in finished quality. In addition, our research group analysed about 3 Mb of new human full-length coding cDNAs from various organ- and development-specific libraries. Recently, we successfully finished the shotgun phase of analysis of several complete bacterial genomes. Moreover, within the framework of the German National Genome Research Programme (NGFN), we have analysed selected regions of the rat genome and chimp chromosome 22.

New technology To support the performance of the lab, the group has ongoing activities in technology development: The robotic environment for DNA preparation and subsequent automatic sequencing has been substantially enhanced, mainly thanks to in-house developments. A complete, colour-based software environment for image analysis was developed, based on the principles of signal theory, and capable of analyzing complex, even disturbed, images and identifying various classes of objects, more exactly than currently feasable. Based again on signal theory, we have developed an entirely novel form of bioinformatics technology for the analysis of information-carrying biomolecules. The advantages: analysis is based on physico-chemical properties rather than on letter code similarities or letter code frequencies and, hence, may shed "new light on old problems". It is possible to combine simple questions to complex, multidimensional questions, virtually without any speed loss. It runs on low-cost hardware – such as simple, Intel-based computers. We are determined to develop the technology further and apply it to the comparative analysis of proteins and DNA, to pattern recognition in complex images, and to the modeling of infection processes in near real-time.

General scheme for the comparison of DNA sequences using a detection filter



02 Modelling of Regulatory Pathways

PROJECT LEADER | **Prof. Dr. Edgar Wingender*** | **Research Group Bioinformatics**

PROJECT MEMBERS | Dr. Torsten Crass | Frank Gössling | Dr. Ines Liebich | Dr. Holger Michael | Dr. Anatolij Potapov | Tilman Sauer | Dr. Klaus Seidl | Ekaterina Shelest

The Research Group Bioinformatics has focussed on the development of computer-aided methods for modelling regulatory networks through the integration of signal transduction and gene regulatory processes.

DHGP2-Project In the course of the BMBF-funded German Human Genome Project (DHGP2), new methods for the formal description of signal transduction pathways and regulatory networks were developed, as well as a computer system for modelling regulatory and metabolic networks on distinct levels of biological organisation. This PheGe (phenotype-genotype) system is designed to provide a platform for linking genotypes with molecular and clinical phenotypes. As a practical example, the collection of data on the genetic and molecular basis of diabetes type II (MODY) and its clinical appearance was chosen – together with our collaborators at the Universities of Magdeburg, Köln and Tübingen/Reutlingen and at the GSF in Neuherberg

Promoter analysis In the context of the BMBF initiative, Intergenomics, we contributed to the elaboration and refinement of methods of analysis, using bioinformatics, of gene promoters involved in immune reactions against infection with *Pseudomonas aeruginosa*. For this, a procedure was developed which can help clarify the biological context of gene expression data obtained from microarray experiments.

Development of databases The systematic annotation of known regulatory elements in the yeast genome and the transcription factors that interact with them were investigated in the context of the EU-funded Comprehensive Yeast Genome Database (CYGD) project. From this work, an independent and publicly accessible database arose, the structure of which was adopted from the well-known database TRANSFAC©. This new information resource is called TSM, TRANSFAC Saccharomyces Module. It can be accessed on the Internet at http://transfac.gbf.de/homepage/databases/tsm/index.html. Within the framework of the BMBF-funded Helmholtz Network for Bioinformatics (HNB), other database projects have also been carried through, including S/MARt DB, a database for scaffold/matrix attached regions of eukaryotic genomes, and ReAlSplice, a database for regulated alternative splice sites and the splice factors acting on them.

Moreover, our Research Group is responsible for the central WWW services of the HNB. In this context the usual, tool-oriented bioinformatics service was complemented by a user-friendly task- and problem-oriented approach to bioinformatics applications.

In collaboration with other partners, a number of "task cascades", which guide the user through a series of programmes, were defined. They run on various servers of the participating institutes and produce an integrated output of results.

* Dr. E. Wingender has taken a C4-Professorship for Bioinformatics at the Georg-August-University Göttingen
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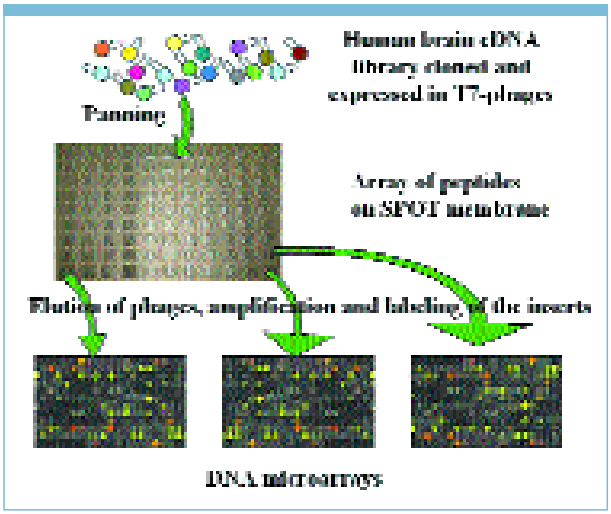
03 Ligand-based Target Discovery

PROJECT LEADER | **Dr. Ronald Frank** | **Research Group Molecular Recognition**

PROJECT MEMBERS | Ulrike Beutling | Krzystof Bialek | Dr. Antonius Dikmans | Varsha V. Gupte | Dr. Jutta Niggemann | Dr. Rene Rübenhagen | Andrzej Swistowski | Dr. Werner Tegge

Random and directed mutagenesis, as well as mRNA inactivation by antisense or RNAi methods, are the classical experimental approaches of forward and reverse genetics for disturbing the function of genes in the analysis of their phenotypic expression. In the last few years, an attractive and complementary new approach has been developed, utilizing synthetic chemical compounds to act directly on gene products – mostly proteins – through the binding of activating or inhibiting ligands. When based on diverse and competent compound repertoires, such a strategy of chemical interference is as globally genomic and systematic an approach as the mutant or antisense/RNAi screening of molecular genetics.



Process for the genome-wide mapping of protein-peptide interactions.

Chemical interference The concept of chemical interference in functional genomics implies that a selective ligand can be identified for almost every gene product, or, more precisely, for every functional binding site they possess. The feasibility of the concept rests on the success of combinatorial synthesis and screening methods that have delivered high affinity ligands for many complex biological targets by empirically searching through vast chemical compound collections. Combinatorial chemistry and functional genomics are thus brought together to help develop new experimental approaches.

Brain specific protein-protein interactions Within the framework of a joint project funded by the BMBF, new biochip technologies for functional proteome analysis are being developed and applied to investigate the human brain. The aim is to establish an automated process for the genome-wide mapping of interactions between protein domains and synthetic peptide ligands which is entirely based on miniaturized high-throughput methods. A cDNA expression library, made from brain mRNA and cloned into a protein-presenting bacteriophage, provides the protein domains. Peptide ligands are chemically synthesized as arrays of 10^3 to 10^6 elements on membrane supports. Following multiplexed affinity enrichment, peptide-specific phages are eluted from the array, amplified and identified by DNA microarray hybridization. We expect to obtain a large net of data for brain specific protein-protein interactions and potential targets for pharmaceutical drug development.

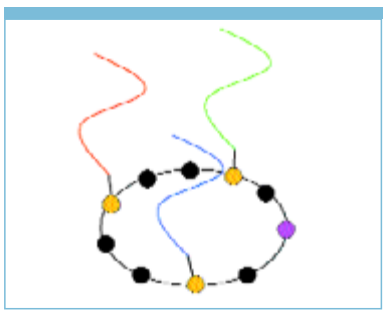
Combinatorial chemical libraries, based on the privileged scaffolds of natural products, are also being developed. Such libraries are intended for internal screening projects but, are also made available to external partners from the disease-oriented genome networks in the NGFN. Thus, initiated by the GBF, we operate a central chemical synthesis unit for a NGFN Chemical Genomics platform.

04 Conformational Protein-Ligand Interactions

PROJECT LEADER | **Dr. Jutta Eichler** | **Research Group Conformational Protein-Ligand Interactions**

PROJECT MEMBERS | Numan Akyol | Dr. Christian Doll | Raimo Franke | Cornelia Hunke | Enge Sudarman

Essentially all biological processes are based on specific binding events, which are initiated by molecular recognition between biomacromolecules, such as proteins – receptors, antibodies, enzymes – and their ligands – antigens, hormones and substrates. The systematic study of molecular recognition phenomena on the molecular level is therefore an important element in the structural understanding of these binding events. The design and generation of synthetic molecules capable of mimicking defined binding and functional sites of natural proteins, represents a promising strategy for the exploration and understanding of protein structure and function. In addition to their basic significance for our understanding and control of protein-ligand interactions, such synthetic proteinmimetics are also useful tools for a range of biomedical applications.

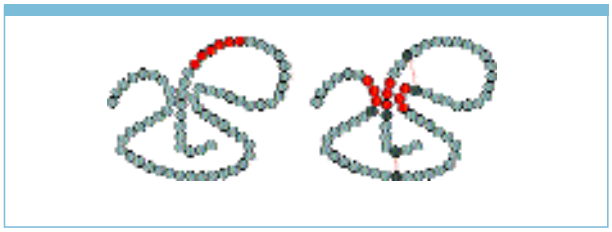


Cyclic peptides as scaffolds for scaffolded peptides. Black: spacer amino acids for the variation of the ring size. Yellow: diamino acids as site-selectively addressable attachment points for peptide fragments. Red, green, blue: Protein-derived peptide fragments making up a discontinuous protein binding site.

Biomimetic synthesis The binding sites of numerous biomedically relevant proteins are generally not located in continuous, consecutive stretches of the amino acid sequence, but rather, in parts of the protein that are widely separated in the amino acid sequence, but brought into spatial proximity by protein folding. The overall objective of this research is to develop and implement a general concept for the synthetic mimicry of such sequentially discontinuous protein binding sites. Such molecules are devised either through "rational design", based on the known structure of the binding site, or through the screening of specifically designed combinatorial libraries of scaffolded peptides, in which peptide fragments are presented through a molecular scaffold in a nonlinear and discontinuous fashion.

The repertoire of synthetic methods developed so far enables the synthesis of structurally diverse scaffold molecules with varying degrees of conformational flexibility. The scaffolds are cyclic peptides with ring sizes ranging from 13 to 33 atoms, which were obtained by incorporating spacer amino acids with varying backbone length. Orthogonally protected amino groups serve as site-selective, addressable attachment points for up to three different peptide fragments.

The current targets are protein-ligand interactions whose structures and binding specificities are well understood, which includes the discontinuous binding site of the EVH1 domain of Mena for proline-rich peptide ligands, the interaction of the bacterial surface protein internalin A with the host cell receptor E-cadherin, the interaction of viral interleukin-6 with the receptor gp130, as well as the discontinuous binding site of the viral envelope protein gp120 for the CD4-receptor on T cells.



Sequentially continuous (left) and discontinuous (right) protein binding sites (amino acid residues contributing to the binding site are marked in red).

05 Comparative Structural Analysis of Metabolic Pathways

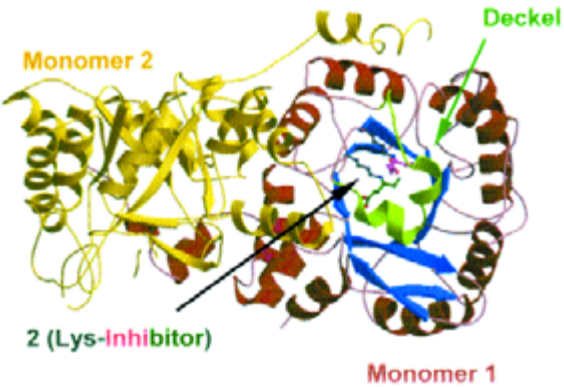
PROJECT LEADER | **Prof. Dr. Dirk Heinz** | **Department of Structural Biology**

PROJECT MEMBERS | Isabell Astner | Dr. Wolf-Dieter Schubert | Jörg Schulze

Far-reaching similarities between human and bacterial metabolism support the Darwinian hypothesis of a common evolutionary origin of all forms of life. Studies into bacterial biochemistry thus commonly reveal a simplified version of more complex human metabolic pathways. Specific differences, however, often provide opportunities to exploit bacterial vulnerabilities by developing specific antibiotics with potentially minimal side effects for human patients.

Tetrapyrroles, such as hemes, (bacterio-)chlorophylls and vitamin B_{12}, are essential constituents of all living cells. They constitute vital cofactors for numerous enzymes and participate in energy and electron transfer processes in photosynthesis and respiration, as well as being involved in transporting oxygen in the blood. The first common precursor of all tetrapyrroles is porphobilinogen (PBG), produced by porphobilinogen synthase (PBGS) through the asymmetric condensation of two molecules of aminolevulinic acid (ALA). Though ubiquitous, PBGSs from different groups of organisms vary significantly with respect to amino acid sequence and especially their metal ion requirements.

In previous work we elucidated the high resolution crystal structure of PBGS from *Pseudomonas aeruginosa*. However, both this and additional crystal structures did not fully clarify the enzymatic mechanism of PBGS. We have now co-crystallized PBGS from *Pseudomonas aeruginosa* with the substrate-like inhibitor 5-fluorolevulinic acid. For the first time, this crystal structure reveals PBGS binding to two substrate-like inhibitors, each of which occupies a specialized pocket at the active site. Each is bound covalently to a lysine residue through a Schiff base. One of the inhibitors is forced into a largely planar conformation. The overall arrangement of PBGS and the inhibitors thus resembles a reaction intermediate, allowing the reaction mechanism to be precisely formulated. A metal ion, also bound at the active site, appears to have the function of aligning the cofactors correctly. To clarify the metal dependency of PBGSs from different organisms further analyses are presently in progress.



Crystal structure of a functional PBGS-dimer. Monomer 1: blue, red and green, monomer 2: yellow. Two inhibitor molecules (red and green ball-and-stick representation) bound to two lysine residues through Schiff bases occupy the active site.

06 Modelling and Analysis of Metabolic Networks

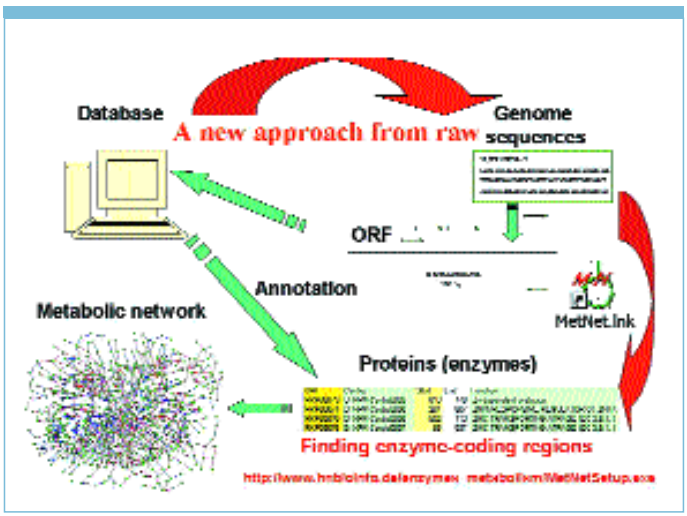
PROJECT LEADER | **Priv.-Doz. Dr. An-Ping Zeng** | **Department of Genome Analysis**

PROJECT MEMBERS | Marcio Rosa da Silva | Eun-Jin Kim | Dr. Hong Wu Ma | Dr. Wael Sabra | Jibin Sun

A gene-enzyme-reaction database was extensively revised during this project. Based on this database and genome information, the metabolic networks of 81 organisms were reconstructed *in silico*. A physiologically more meaningful definition of metabolic path length was developed to characterize the hierarchical and functional organization of metabolic networks. The average path lengths of the networks were then calculated and compared for all the organisms. In contrast to recent reports in literature, our research group found that eukaryotes and archaea generally have longer metabolic path lengths than bacteria, indicating quantitative differences in the structure and evolution of metabolic networks.

Fast reconstruction of metabolic pathways A new and fast method for *in silico* reconstruction of metabolic networks directly from raw genome sequences was developed. Instead of using ORFs to query public databases, entries from public DNA and protein databases are used as queries to search a local database for raw genome sequences of an organism to identify ORF similar regions (ORF-SRs) that encode proteins. The well-annotated genome of *Salmonella typhimurium* LT2 was used as an example to demonstrate the applicability of the method. 99 % of the reported 1050 ORFs were identified as enzymes with an EC number and assigned the same functions using the SWISS-PROT and TrEMBL databases. Furthermore, two versions of raw genome sequences with different genome coverage – 3.9-fold and 7.9-fold respectively – from the bacterium *Klebsiella pneumoniae* were compared in order to identify OFR-SRs. 98.9 % of the ORF-SRs identified with the 7.9-fold genome sequences were also found with the 3.9-fold genome sequences, suggesting that with our approach a 3.9-fold sequence coverage of the genome can be used for the *in silico* reconstruction of metabolic network, at least with this organism. The new method permits accelerated genome-wide metabolic comparison of different organisms.

Modeling of metabolic and genetic networks
In this subproject of the BMBF project 'Intergenomics', mathematical models are being developed for the analysis and simulation of metabolic and genetic networks involved in the formation of virulence factors and stress responses related to *Pseudomonas aeruginosa* (PAO1). For this purpose, *P. aeruginosa* was cultivated under defined physiological conditions to generate the required data. We found a possible new defense mechanism of this pathogen against reactive oxygen species. Mechanisms discussed so far in the literature mainly include the production of certain enzymes, such as catalase, and the formation of biofilms. For the first time, we showed that this pathogen can strongly reduce the transfer of oxygen from the gas to the liquid phase, thus causing oxygen-limitation in the culture and blocking the O_2 source for the formation of reactive species. Under these conditions, *P. aeruginosa* grows better and the formation of some virulence factors, such as elastase, is strongly enhanced.



Reconstruction of metabolic networks from genome information. The blue arrows show the conventional way by using annotated genome sequences through ORF prediction. In a new approach (red) we reversed the query process and simplified the annotation process by just finding the enzyme-coding regions.

Programme "Sustainable Use of Landscapes"

PROGRAMME SPEAKER | **Prof. Dr. Kenneth N. Timmis** | **Department of Environmental Microbiology**

- Microorganisms are ubiquitous and, because they can tolerate environmental conditions far too extreme for higher organisms, their habitats define the biosphere. Microbial activities profoundly influence both global processes, *e.g.* the carbon cycle and global warming, and local ones, *e.g.* they cause disease in plants and animals; provide essential nutrients for plants and animals. Microbes critically impact human beings and their activities positively and negatively in a multitude of ways: some are responsible for the greater portion of human disease and mortality, whereas others provide us with antibiotics to treat disease, and yet others play a critical role in cleansing our environment of organic wastes. Much of biotechnology is based on microbes and their products. Our ability to influence microbial activities, in order to obtain greater benefit from positive ones and to diminish the effects of negative ones, requires an understanding of how microbes live and function in their habitats, and how their activities are regulated.

Classical microbiology focuses on the study of pure cultures growing under laboratory conditions. However, microbes in nature grow as complex, diverse and dynamic communities, the members of which interact and share available resources in complex ways. It is these interactions, and interactions with other biotic and abiotic components of their environment, that determine community activities. At present we have no general understanding of such interactions.

The goals of the Environmental Biotechnology research programme are to understand microbial communities as functional units, to elucidate the critical interactions that regulate community activities, to develop and validate interventions that result in substantive increases in activities of biotechnological interest, and to discover new microbial products and metabolic activities by exploring microbial diversity. A multi-scale (gene, organism, community; test tube, chemostat, natural habitat) and multi-disciplinary (microbial ecology, physiology, phylogeny, biochemistry, analytical chemistry, genetics/genomics, bioinformatics, and modelling) approach characterises the research programme. Though the results obtained will be generally applicable to most types of microbial community, our research focusses on microbial communities that metabolise environmental pollutants, and an important goal of the programme is to make key contributions to the sustainable development of our society.

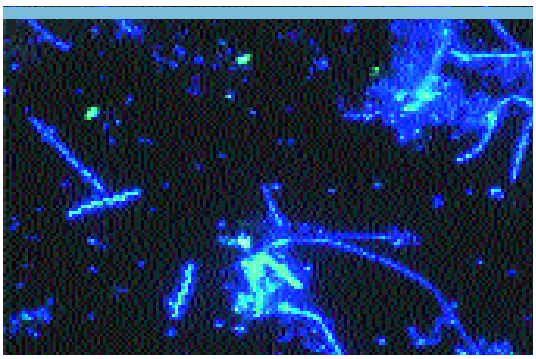
01 Functional Genomics and Niche Specificity

PROJECT LEADER | **Prof. Dr. Kenneth N. Timmis | Department of Environmental Microbiology**

PROJECT MEMBERS | Dr. Andreas Felske | Olga Golyshina | Filip Kamenski | Dr. Matthias Labrenz | Dr. Alexander Neef | Daniela Regenhardt | Dr. Vitor Dos Santos | Massimo Strocchi | Dr. Roland Weller | Dr. Dirk Wenderoth

In collaboration with TIGR (USA), and QIAGEN, DKFZ (Deutsches Krebsforschungsinstitut) and the MHH (Medizinische Hochschule Hannover), the genome of the *P. putida* KT2440 safety strain has been sequenced, anno-tated and its particular genome features analysed. This investigation has revealed an unusually large number of genes whose products are involved in transcription regulation, transport, environmental signalling and catabolism. These findings are consistent with a highly versatile metabolism and reflect the emphasis in *P. putida* on powerful cellular mechanisms that enable it to thrive in diverse environments and to compete successfully with other organisms.

Genomic comparisons between the saprophytic KT2440 strain and plant and animal pathogenic strains of *Pseudomonas* have shown that KT2440 lacks a spectrum of key virulence determinants that mediate host damage, including exotoxins, specific hydrolytic enzymes, type III secretion systems and factors mediating hypersensitive responses. Genetic determinants that are shared between KT2440 and pathogenic strains of *Pseudomonas* suggest that certain properties, may in fact only be important for effective colonization and survival on surfaces, and not obligatorily related to pathogenesis. This genomic analysis has thus confirmed the avirulence of KT2440, provided a definitive genetic basis of the biosafety characteristics of this bacterium, and generated a database upon which the environmental and biotechnological behaviour of this strain can be interpreted.



- *To study bacteria in the environment, culture independent processes are used, as most of the bacteria cannot be cultivated in laboratory until now. Cloning of the whole DNA of samples from the environment in mega genome libraries allows to access also non-cultivatable bacteria biotechnological processes.*

Photo: GBF

***P. putida* and *P. aeruginosa* – a comparison** *In silico* metabolic models describing genotype-phenotype relationships for *P. putida* and *P. aeruginosa* have been derived on the basis their genome sequences, biochemical knowledge and strain-specific information. A comparison of the metabolic space of the central metabolism of these bacteria shows that the number of elementary pathways that represent the metabolic potential of *P. aeruginosa* is 2 to 6 times higher than those for *P. putida*, although the former has only two more reactions than the latter. This reflects a higher flexibility of the central metabolism of *P. aeruginosa* as compared to *P. putida*. This is clearly an emergent property of the system that could not be predicted solely on the basis of a linear comparison of gene lists.

A proteome map of KT2440 has been constructed and its proteome responses to several environmental signals, like adhesion to surfaces, iron deprivation and water stress, have been analysed. A large number of differentially expressed proteins have been identified. A library of mini-transposon mutants has been generated and used to test hypotheses and predictions about the ecophysiological behaviour of *P. putida* that emerge from *in silico* genome analyses.

In the Genomic Network Bielefeld, the genomic sequence of *Alcanivorax borkumensis* – a cosmopolitan oligotrophic –is currently being determined, a mutant library generated, and its proteome analysed. Once annotation has been completed, an *in silico* metabolic model similar to that for the *Pseudomonas* strains discussed above will be developed. Comparison with the *P. putida* genome – a cosmopolitan copiotrop – will suggest key functions that may account for the different lifestyles, and that can be experimentally analysed.

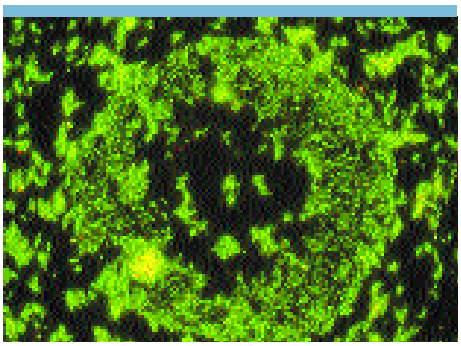
02 Biofilm Communities in Environment and Health

PROJECT LEADER | **Dr. Wolf-Rainer Abraham | Research Group Chemical Microbiology**

PROJECT MEMBERS | Wanda Fehr | Dr. Heidrun Jungnitz | Dr. Heinrich Lünsdorf | Stefanie Tillmann | Prof. Dr. Kenneth Timmis | Dr. Irene Wagner-Döbler | Dr. Dirk Wenderoth | Robert Witzig

A complex biofilm community originating from contaminated soil was cultivated on droplets of polychlorinated biphenyls (PCB), from which various *Burkholderia* strains were isolated. These isolates were capable of degrading low-chlorinated PCB congeners, whereas the PCB-biofilm community degraded tri- and tetra-chlorinated congeners as well. To identify the organisms responsible for the degradation of specific PCB congeners in the biofilm, ^{13}C-labelled congeners were introduced into the PCB mixture in microcosm experiments. Incorporation of ^{13}C into the biomass was monitored using isotope ratio mass spectrometry (IRMS). In order to distinguish different members of the biofilm, taxa-specific compounds had to be identified. A procedure to isolate taxa-specific 23S rRNA by binding it to specific probes was developed, however 23S rRNA proved to be a target molecule with a very slow rate of isotope incorporation. The fatty acids of phospholipids from the same experiment were analysed and found to have good rates of ^{13}C incorporation. The fatty acids labelled by ^{13}C incorporation from a trichlorinated congener were the same molecules found in the *Burkholderia* isolates, thus demonstrating that *Burkholderia* species are the degraders of this congener. Using stable isotope tracers, it could be shown that the multi-species biofilm is able to degrade compounds which are not attacked by its isolated members. To get a broader insight into the metabolic potential of the biofilm community, the metagenome of the PCB-biofilm was cloned and is currently being analysed in the project Functional Genomics.

Stent biofilms A recurrent problem with bilary stents, after their implantation, is the growth of bacteria, which form thick biofilms, finally blocking them and causing severe problems for the patient. Stents from different patients in Braunschweig were collected and the microbial biofilm community compared with stents obtained from Kiel and Italy. The different microbial communities showed bacteria common to all stents, with small variations in other community members. Stents from the same region possessed biofilm communities more closely related than to those from other regions. Biofilms grown from stent biofilms in microcosm experiments revealed a pronounced succession of colonizers. The initial colonizer was always *Pseudomonas aeruginosa*, which has also been found in all stent biofilms so far analysed. The results point to the bilary fluid as one of the main factors shaping the microbial community.



Biofilm grown on PCB micro-droplets. Bacteria stained green are alive, those in yellow are damaged and the few in red are dead. Note the different types of bacteria (e.g. the cluster in the centre).

Photo: GBF

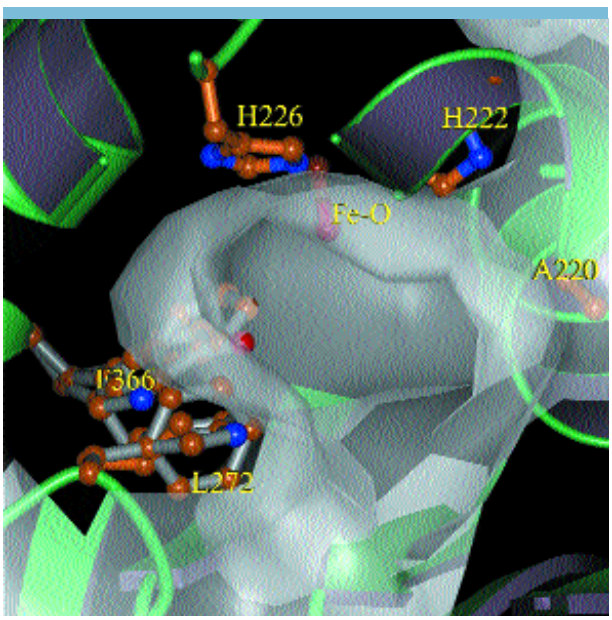
03 Metabolic Diversity

PROJECT LEADER | **Priv.-Doz. Dr. Dietmar Pieper | Research Group Biodegradation**

PROJECT MEMBERS | Dr. Hans-Adolf Arfmann | Wera Bode | Dr. Heike Görres | Dr. Bernd Hofer | Howard A. Junca | Anna-Maria Kicinska | Patricia Nikodem | Dr. Peter Rapp | Carsten Strömpl | Sabine Wittrock

Mikroorganisms are characterized by their broad metabolic capabilities and their flexibility in adaptating to new environmental conditions. Microbial metabolism comprises a net of interactions between microbial community members, with most of them not being cultivable using methods currently available. For the characterisation of metabolic networks, a detailed understanding of metabolism in pure cultures and model communities is necessary, as well as the availability of culture-independent methods for analysing complex microbial systems.

Our work has concentrated on the network involving the metabolism of hydrophobic aromatics. In order to study the structure-function relationship of ring activating dioxygenases, key enzymes initiating the degradation of various pollutants, genes from novel bacterial isolates have been analyzed, hybrid genes produced, and also site-directed mutants generated. Site-directed mutants with new regio-selectivity for the initial attack on chlorotoluenes and polychlorinated biphenyls were obtained. Sequence comparisons permitted the identification of sub-regions critical for the interaction between substrate and active site.



Model of Tetrachlorobenzene dioxygenase indicating the influence of mutations in positions 272 and specifically 366 on the shape of the substrate binding pocket.

Enzyme activities in balance *Pseudomonas* MT1 originates from a chlorosalicylate degrading community, where complex metabolic interactions make the mixed culture more efficient at degradation than single members. The metabolism of chlorosalicylate by MT1 was previously assumed to involve protoanemonin, a highly toxic substance, as an intermediate. The group was able to elucidate the metabolism and characterize how protoanemonin formation is prevented. Such prevention necessitates the close collaboration between two key enzymes, one of which acts on an unstable intermediate formed by the other. Evidently, chloroaromatic degradation necessitates a well-balanced network of enzyme activities, obviously recruited from various pathways for aromatic degradation, which only occasionally occur in one and the same organism. Even MT1 excretes metabolic end-products, such that degradation evidently will be better managed by complex microbial communities.

Chloroaromatics from various environments The potential for the degradation of chloroaromatics in various environments was assessed by community structure profiling and characterization of catabolic gene abundance using PCR-amplification employing primers specific to three classes of key catabolic genes. Community structure profiling during biostimulation showed different communities arising. However, all stimulated cultures were dominated by organisms exhibiting chlorocatechol 1,2-dioxygenase activity. This was verified by genetic means and by a new metabolic test for the whole community.

Thus, assessment of community function necessitates functional gene profiling rather than community structure validation. Both, Pseudomonads isolated directly from samples, and *Acidovorax* strains isolated after stimulation, showed a positive amplification reaction with the chlorobenzene dioxygenase and chlorocatechol 1,2-dioxygenase specific primers. This indicated the presence of a classical chlorobenzene metabolic pathway in these isolates, which also seemed to be responsible for degradation under environmental conditions.

04 Natural Products

PROJECT LEADER | **Prof. Dr. Kenneth N. Timmis | Department of Environmental Microbiology**

PROJECT MEMBERS | Dr. Tatiana Chernikova | Wanda Fehr | Dr. Manuel Ferrer | Dr. Christa Hoch | Dr. Rolf Jansen | Dr. Gabriella Molinari | Dr. Björg Pauling | Magally Romero-Tabarez | Dr. Irene Wagner-Döbler | Kerstin Wilke

While, on the one hand, the resistance of pathogens against commercial drugs is still increasing, on the other, the diversity of micro-organisms is enormous and still only a very tiny fraction of environmental isolates has been screened for their production of bioactive compounds. To exploit this huge amount of untapped microbial biodiversity, novel environmental isolates are screened in this project for their production of bioactive compounds, in order to find new drugs with new mechanisms of action.

A new metabolite from 140 extracts During the first phase of the study, 140 extracts with biological activity were selected for characterization. Of these, 78 exhibited antibacterial and antifungal activities, and 27 showed cytotoxic activity towards human cells. A number of the isolates did not consistently produce bioactive compounds after sub-cultivation, so efforts were made to define cultivation conditions that favour production of the compound initially produced. Bioactive principles were purified and analysed by HPLC-UV-MS, and their spectra compared with those of known structure (and available as data bases), in order to identify known compounds. 55 substances were identified as known variants of Actinomycin, Amicoumacin, Bacillaene, Bacyllomycin, Bogorol, Difficidin, Fungichromin, Filipin, Moenomycin and Quinolones in extracts showing antibacterial and antifungal activities. Several antibacterial peptides are currently being characterized. A potentially new metabolite that belongs to the group of macrolactins is also currently being characterized. Recognized known compounds that demonstrate new activities, particularly against vancomycin-resistant Enterococci and *Pseudomonas aeruginosa* and *Burkholderia cepacia*, require additional studies to re-evaluate their structures and to assess their utility.

New herbicidal and insecticide substances Chlorophyll fluorescence analysis of plant growth in the presence of the extracts identified six herbicidal substances, which are currently being characterized. 100 extracts were also tested against *Anopheles albimanus*, a mosquito vector of human diseases, *Spodoptera frugiperda* and *Tenebrio molitor*, two agronomically relevant pest insects and *Musca domestica*, a biological vector for several infectious diseases. Twenty of the tested extracts showed larvicidal activity against *Anopheles albimanus* and eight showed insecticidal activity against *Musca domestica*.

Extreme locations like these hot springs on the Chilenean Altiplano are habitats for unusual bacteria. Here, unknown bacteria can be found, from which new active compounds may be isolated.

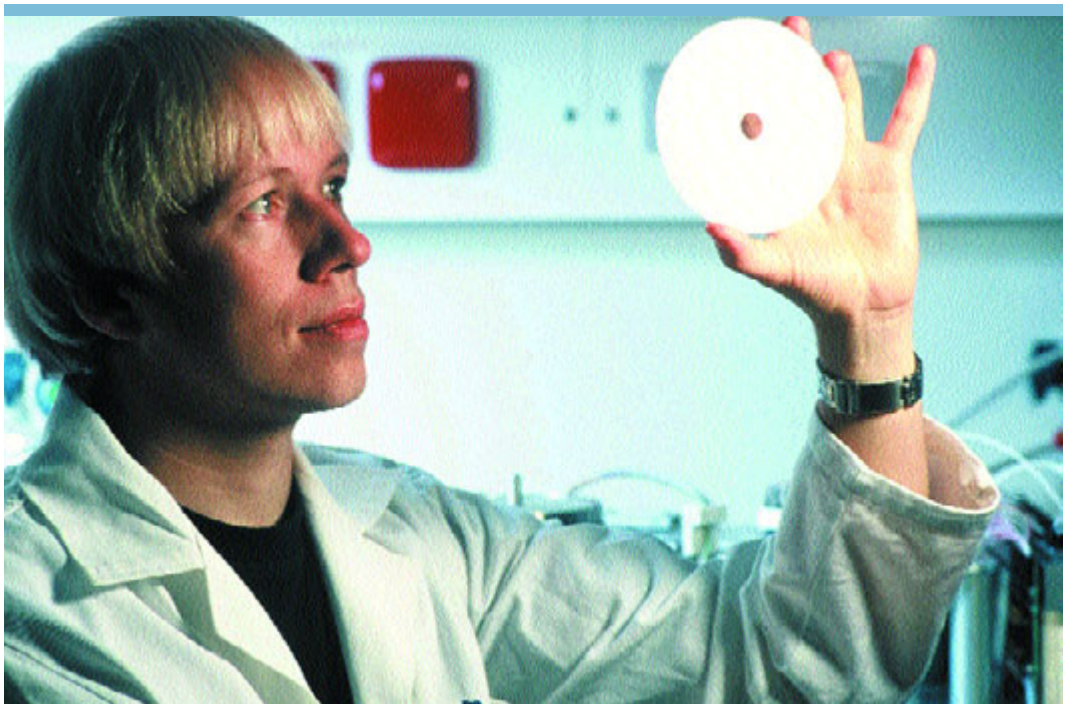
Photo: Prof. Chong, Universidad Católica de Norte, Antofagasta, Chile, and Abraham, GBF

To evaluate the possibility of using *C. elegans* as a test system to identify and characterize new compounds, 20 extracts selected from the primary screening were tested for activity against this nematode. Five of the extracts killed the worm and will be further characterized.

An important aim of the project is to identify substances that have different mechanisms of action from those of drugs currently in use. We will thus set up screens to identify compounds that are able to inhibit critical steps of the pathogenesis process, such as bacterial attachment and/or microbial capacity to produce biofilms, which may provide important alternative chemotherapeutic strategies.

Technological Platforms

A number of platform technologies essential for the research carried out at the GBF are made available to the scientific projects as centralised facilities. In the context of national and international research programmes, these platforms provide services not only to internal projects, but also to scientific collaborators from other Helmholtz research centres, from German universities and from other public research institutes, as well as from industry. Below, some of the most important platforms are described in detail.



Our internal synthesis service also uses processes that have been developed in the GBF. Here, Dr. Norbert Zander evaluates the quality of an injection-moulded polypropylene compact disc, which will be used as carrier for the preparation of peptide arrays.

Photo: Bierstedt

01 Central Animal Facility

HEAD | **Dr. David Monner | Central Animal Facility**

SCIENTIFIC COLLABORATOR | Dr. Werner Müller

The purpose of the Central Animal Facility is to care for and provide research animals – principally mice – for the scientists at the GBF and monitor compliance to the guidelines of the federal Animal Welfare Act. In addition to caring for breeding colonies, both under specific pathogen-free (SPF) conditions and in quarantine, our activities include performing back crosses and experimental breedings to create new mouse lines, rederivation of strains by embryo transfer, archiving strains by embryo cryopreservation, maintenance of nuclear breeding colonies, and breeding and provision of donor animals and pseudopregnant females for the generation of new genetically-modified mouse lines by blastocyst injection of ES-cells.

Moving and controlling of mice

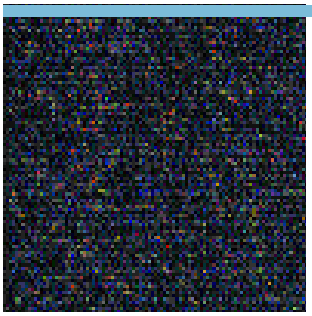
Photo: Bierstedt

Services In 2002, cage occupation in the facility approximately doubled to almost 2000 by the end of the year. Currently, over 100 different mouse lines are housed in the facility. Besides providing standard animal care, the animal technicians carry out all experimental breedings, with attendant data base administration, and perform a number of services, including biopsies, blood sampling, immunisations and other manipulations. During the course of the year, the basic techniques for manipulating the mouse embryo have been successfully established in the facility. Since September, several mouse lines have been rederived, and since November several lines were archived by embryo cryopreservation. In August, a training programme for laboratory animal technicians was established.

Expansion of the infection platform The animal facility also supervised planning and remodelling of the annex of Building D to a dedicated animal care unit for infection experiments of safety level 2. The unit went into service in September 2003, thus providing sufficient space for the implementation of the infection challenge platform.

02 Gene Expression Analysis

HEAD | Dr. Jörg Lauber †, Dr. Robert Geffers | Research Group Mucosal Immunity



Read-out of an Affymetrix array which contains about 400,000 oligonucleotides synthesized on a glass surface

The basic methodology for the serial production of Affymetrix Arrays does not only allow large-scale parallel analysis of the expression of thousands of genes, but also – thanks to their reproducibility – the comparison of experiments performed at different times. It is possible to investigate up to 40,000 genes with respect to their expression from the human, mouse and rat genome. Customers of the GBF Array Facility – GBF reseach groups, universities, hospitals and other research facilities – have approximately 500 genes of human or murine origin at their disposal, to verify array data by real-time PCR. In this context, the GBF Array facility also provides a service to identify new oligo-primers for genes which are currently not available.

Bioinformatics support The increasingly complex structure of experiments requires software and algorithms capable of analysing the large amount of data produced. Thus, a complete series of "cluster software" is being used, allowing us to find groups of regulated genes which correspond, for example, to a certain signal pathway. For this, it is also important to consider the so-called GO (gene ontology) annotations, which allow classification of the regulated genes according to function and sub-cellular localisation. In order to clarify signal paths and functional correlations, software is used which displays expression data graphically at the protein level.

An Access database was programmed and built which not only allows access to all data produced in the Array Facility, but which also starts the corresponding software along with the respective data. Furthermore, a process can be initiated that automatically compiles experimental data according to the MIAME format (Minimal Information About Microarray Experiments).

Research themes The research themes of the individual arrays varies, according to customers and cooperation partners, covering various topics of biology and medicine. Most of the work concerns experiments related to host-pathogen interaction and the host immune response.

In the area of host-pathogen interactions, diverse pathogens, such as EHEC, Mycobacteria, *Salmonella*, *Chlamydia*, *Yersenia* or *Lysteria*, were cultivated *in vitro* in different cell lines, which correspond to the different target tissues of the pathogen. In addition, model organisms – mice in this case – were infected with bacterial pathogens, and, subsequently, different tissues analysed.

In the area of immunology, T cells with regulatory properties were investigated, including $CD4^+CD25^+$, $CD4^+CD45^{low}$ or anergic cells, from different transgenic mouse models. These regulatory cells are important for autoimmunity, but also for the regulation of the immune response in the case of an infection. Different cell types of the immune system were isolated by cell sorting and their expression patterns compared, in order to find genes implicated in regulatory cascades. In this way, a series of genes were found which affect the regulatory programme. Currently, several of these genes are being investigated in more detail in overexpression and "knockout" experiments.

† Dr. Lauber died in May, 2003. He was a highly recognized scientist who established the Expression Array Facility.

03 Analytical Instruments

HEAD | **Dr. Victor Wray** | **Research Group Biophysical Analysis**

SCIENTIFIC COLLABORATORS | Dr. Heinrich Lünsdorf | Dr. Manfred Nimtz | Dr. Manfred Rohde

This platform is a facility for determining the three-dimensional structure of all types of natural products and is equipped to carry out mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), X-ray crystallography, protein sequencing, electron microscopy and confocal laser microscopy. For the majority of low-molecular natural products, the total structure is elucidated in a routine manner using a combination of MS and NMR spectroscopy.

The direct analysis of large, intact biomolecules, such as proteins, oligonucleotides and complex carbohydrates is routinely carried out using MALDI- and ESI-MS. Mass spectrometry has the important advantage of providing information about very small amounts of compound. The secondary and tertiary structure of peptides and proteins can be elucidated in solution, when appropriately labelled material – ^{15}N and ^{13}C – is available, through the application of multidimensional NMR spectroscopy. Emphasis in the macromolecular field has been placed on MS-elucidation of glycoproteins, in particular the characterisation of oligosaccharides using MALDI and ESI-MS/MS techniques and hydrolytic micro-derivatisation methods.

Dr. Victor Wray controls a sample for a NMR-measurement

Photo: Bierstedt

The automatisation of MS-micro-techniques for the identification and characterisation of proteins from 2D gels through determination of the molecular weight of their proteolytic fragments using HPLC ESI-MS/MS has been established.

X-ray crystallography The main emphasis in X-ray crystallography is the structural analysis of proteins at the atomic level. A pipette-robot, as well as a modern X-ray unit with an area detector and rotating anode, are available for crystallisation and data collection. Privileged access to synchrotron radiation at DESY in Hamburg allows the generation of high resolution data and phase determination using anomalous dispersion.

Edman degradation N-terminal protein sequencing is performed by automated Edman degradation. Applications include the elucidation of new protein sequences, the identification of proteins in data bases, as well as checking the identity and purity of recombinant proteins. Samples, either in solution or bound to PVDF-membranes, may be analyzed in the low picomolar range.

FESEM-techniques Electron microscopy is used to visualize the adherence to and invasion of host cells by a wide range of pathogens. Preparation protocols have been customized to undertake studies using high resolution field emission scanning electron microscopy (FESEM), which have revealed distinct pathways for invading the same host cell. In addition, a methodology has been developed to immuno-localize pathogenicity factors using FESEM, not only on the bacterial cell surface or the interface between bacterial and host cell membrane, but also inside the host cell, using antibodies and colloidal gold-particles.

04 Peptide- and Chemical Synthesis

HEAD | **Dr. Werner Tegge** | **Research Group Molecular Recognition**

SCIENTIFIC COLLABORATORS | Dr. Ronald Frank | Dr. Michael Morr

This platform is part of the research group Molecular Recognition. Synthetic, soluble peptides, arrays of immobilized compounds, and special, commercially not available, compounds are generated in close collaboration with users. For the synthesis, modern equipment is employed. Soluble peptides are routinely characterized using HPLC and MALDI mass spectrometry. If necessary, further characterization is carried out by amino acid analysis, sequencing, special mass spectrometry techniques and NMR in the GBF's Department of Structural Biology.

Depending on intended usage and desired quality of the crude products, purifications are carried out, usually by preparative HPLC. For special investigations, modified peptides are required and synthesized accordingly. Routinely, the platform offers the following modifications: phosphorylations, biotinylations, lipid additions, branched peptides and cyclizations.

SPOT-arrays The synthesis of peptide arrays for the systematic and empirical search for peptide ligands is also carried out in close cooperation and collaboration with users. In particular, for the design of such arrays a thorough understanding of the biological problem is essential, for which a close cooperation with our clients is essential. The SPOT-arrays are generated on paper sheets or other polymeric supports, according to the method developed by Dr Ronald Frank, currently semi-automatically, but in the near future with the help of new synthesis robots, fully automatically. Every year, approximately 15,000 peptides and peptide mixtures are generated in an array format and utilized for the investigation of protein-protein interactions and enzyme-substrate recognition. In a 20-step synthesis, galactosyl ceramide was synthesized and coupled to a synthetic peptide. This class of compounds is used by the GBF's Vaccine Research group for the development of mucosal vaccines.

Until today, the service unit has produced and characterized about 2000 soluble peptides. Modern equipment together with personal knowledge are fundamental for this service.

Photo: Bierstedt

Programme "Biotech Facilities"

PROGRAMME SPEAKER | **Dr. Holger Ziehr** | **Research Group Quality and Product Management**

In 2002, the Biotech Facilities of the GBF were re-orientated to a technology platform providing services for clients both within and outside the Helmholtz Association. These services comprise the development and scale-up of cultivation processes for microbial and animal cells, and purification processes for the isolation of biomolecules, such as proteins, nucleic acids and antibodies from cell mass and supernatant. At the GBF, various biotechnological pilot plants are available for this purpose, housing bioreactors and centrifuges, as well as chromatographic and filtration systems. The facilities have been licensed since 1997 in accordance with the German Drug Act (AMG), thus enabling novel active pharmaceutical ingredients to be produced for clinical research. In compliance to the regulations, a highly compartmented clean room pilot plant (GMP I) was installed in 1999. In order to satisfy increasing demand for capacity and quality, an additional plant was installed in 2001 (GMP II). The commissioning and qualification of GMP II was planned for 2002, but was put on hold temporarily. During 2002, a total of 240 fermentations were performed in the Biopilot Plant, of which 90 were for external clients. Of these, 37 came from universities and academia and 53 from industry.

GMP-quality pharmaceutical ingredient production

Due to the complexity of projects for GMP-quality pharmaceutical ingredient production, the number of such projects is small, whereas the resources required are fairly extensive: Fermentation processes for two vaccine candidates against malaria (MSP-1 (K1), MSP-1 (3D7)) expressed in *E. coli* were developed for the "Zentrum für Molekulare Biologie der Universität Heidelberg" (ZMBH). The cell mass was transferred for further processing to the Walter Reed Army Institute of Research (WRAIR) in Silver Springs, MS, USA. Due to the current difficult economic situation in Germany, the demand for GMP-process development and production from start-up Biotech companies has declined. However, this decline was well compensated for by an increased demand from the pharmaceutical industry. During 2002, process development projects and feasibility studies were carried out. All of these projects focussed on the development of processes for recombinant biopharmaceutical ingredients.

Stefan Kluger controls the 500 L bioreactor of the GMP II-plant.

Photo: Bierstedt

01 Microbial Expression and Production Systems

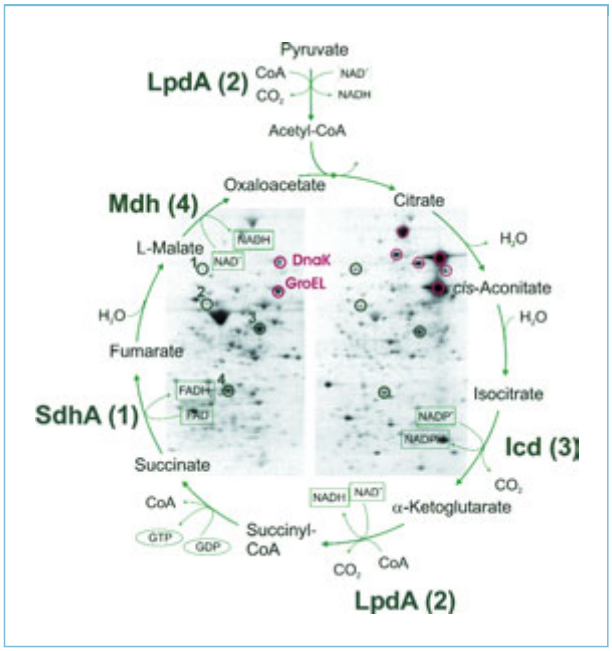
PROJECT LEADER | **Priv.-Doz. Dr. Ursula Rinas | Research Group Downstream Processing (formerly: Research Group Microbial Systems)**

PROJECT MEMBERS | Eriola Betiku | Heike Baars-Hibbe | Luis Felipe Vallejo | Xin Lu

Proteins from recombinant microorganisms Our work is concerned with the identification and resolution of bottlenecks associated with the production of biologically active proteins using recombinant microorganisms. It includes investigating the physiology of protein producing cells and the mathematical description and prediction of cellular reactions to the forced synthesis of foreign protein employing techniques such as proteom and metabolic flux analysis. We are also concerned with developing process strategies for the efficient synthesis, possible renaturation, and purification of biologically active proteins from microbial expression systems

Successful protein production through an understanding of the physiology of the microbial cell factory The energy requirement of the producing cell strongly depends on the environment of the producing organism and the specific properties of the target product. Synthesis of a protein with a tendency to aggregate, or which is prone to proteolytic degradation, may cause cells to respond with an increased energy requirement. This may result not only in increased respiratory activity, but also in reorganisation of the synthesis of metabolic enzymes of the energy generating machinery.

Based on this knowledge, a high cell density procedure for the production of human bone morphogenetic proteins, such as hBMP-2, was developed, which resulted in a yield of 8-9 g L^{-1} rhBMP-2 in the bioreactor. The recombinant protein was produced in the form of inactive inclusion bodies. The applied renaturation and purification procedure resulted in an active protein corresponding to 750 mg biologically active rBMP-2 per liter of culture broth.



Analysis of cellular protein synthesis by two-dimensional gel electrophoresis revealed elevated synthesis rates of cAMP-CRP controlled enzymes of the tricarboxylic acid cycle (1: SdhA succinate dehydrogenase, 2: LpdA dihydrolipoamide dehydrogenase, dehydrogenase subunit of the multienzyme complex of pyruvate dehydrogenase and ketoglutarate dehydrogenase) after temperature-induced product synthesis. (left side: proteom prior to induction; right side: proteom after induction; ^{35}S-labelling). The more abundant dehydrogenases of the tricarboxylic acid cycle (3: Icd isocitrate dehydrogenase, 4: Mdh malate dehydrogenase) show slightly reduced synthesis rates after induction. A pronounced increase in the synthesis of heat shock proteins after induction is obvious (marked in red, DnaK and GroEL are highlighted).

02 Biological System Analysis

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While in the past, our activities were mainly directed towards developing strategies for efficient microbial degradation processes, more recently, our work has been focused more towards the elucidation of biological reaction mechanisms by combining experimental techniques with mathematical modelling. On the basis of mechanistic concepts, mathematical models were generated capable of describing a system's dynamics. The discrimination of different models can help to elucidate reaction mechanisms and pathways.

Protoanemonin formation by *Alcaligenes euthrophus* In collaboration with the research group Biodegradation, a new catabolic pathway of *Alcaligenes euthrophus*, which leads to the formation of protoanemonin from 2-chloromuconate, was investigated. Two enzymes catalyse this reaction, a muconate cycloisomerase, (MCI), and a muconolactone isomerase, (MLI). In addition to protoanemonin, cis- and trans-dienlactone are also formed, and 2- and 5-chloromuconolactone (2CML and 5CML, respectively) were identified as intermediates. The reaction mechanism was elucidated with the aid of a mathematical model. Using the steady state theory, the differential equations of all the involved reactions were generated and the system solved numerically using the Matlab software platform. Experimental results and a good correlation between model and experiments produced a reaction mechanism based on an equilibrium between the substrate and the two intermediates. Protoanemonine is then formed from 2CML, and cis- and trans-dienlactone from 5CML by irreversible reactions. The model is also able to predict the product ratio of *in vitro* kinetic experiments using different enzyme ratios quite well.

Kinetics of multiple substrate systems The design of efficient treatment systems for industrial waste waters, containing toxic compounds, based on reaction kinetic, requires kinetics capable of describing the system under stationary as well as under dynamic conditions, and kinetic equations derived for single toxic compounds, being transferable to multiple substrate systems. The transferability of kinetic equations was investigated using the degradation of phenol, benzoate, and acetate by *Burkholderia cepacia* G4 as a model system. It was shown that mixtures of these three compounds were degraded completely and simultaneously. Under stationary conditions, degradation of substrate mixtures can be well described using the stoichiometric and kinetic parameters derived from single substrate experiments.

The presence of more than one carbon source leads to an increase of the critical dilution rate at which substrate accumulation is observed in the reactor. By this means, the efficiency of the process is increased. Under dynamic conditions, decoupling of the catabolic and anabolic flows was observed. The microorganisms adapted slowly to changing environmental conditions. The time constants revealed that this adaptation is the result of genetic, not enzymaticregulation, most probably by adjustment of enzyme concentration in the cell. Hence, macroscopically, kinetic and stoichiometric parameters are not constant, and "culture history" has an influence on the kinetics.

03 Cell Culture Technology

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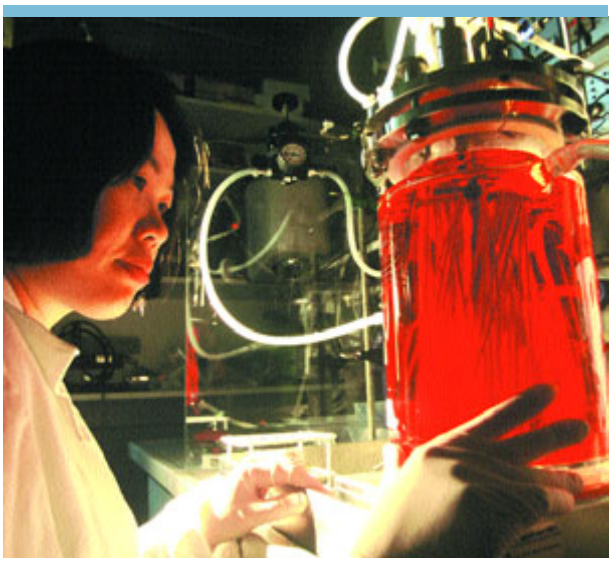
One of the most important challenges in Cell Culture Technology is the reliable and reproducible cultivation of animal cells under completely defined culture conditions. The goal is to eliminate components of animal origin, thereby, as far as possible, making use of new economic and competitive processes.

Optimising the productivity of suspended HEK293 cells Compared to microbial expression systems, protein expression in mammalian cells is time-consuming, mainly because of the difficulty of generating a stably producing cell line. In contrast, transient gene expression without stable integration into the chromosomal DNA enables protein synthesis to proceed immediately after gene transfer. Up to now, transient transfection was characterized by the disadvantage that at least a low amount of serum was necessary for efficient gene transfer.

To optimise the productivity of transiently transfected HEK293s cells, a model production system was established in collaboration with the Department of Gene Regulation and Differentiation, initially by constructing two different bicistronic plasmids expressing the target gene in the first position and a reporter gene in the second, and *vice versa*. Applying polycation-mediated gene delivery, we thus established a transfection system which produced up to 70 % transfectibility under completely defined serum-free culture conditions.



Work in the Cell Culture Lab: Starting a fermentation

Photo: Bierstedt

Perfusion system for mammalian cell bioreactors All cell separation systems have the disadvantage of being limited in their scalability. Therefore, a special hydrocyclone for separating mammalian cell lines in continuously perfused bioreactors was developed.

During a 2-hours, quasi steady state period of HeLA cell cultivation in a 6 L stirred tank bioreactor, the hydrocyclone was operated for 3 min at a flow rate of 1 L min^{-1} This corresponded to a residence time of the culture broth within the hydrocyclone of less than 0.2 s. Viability of the cells was always above 90 %. Such hydrocyclone performance can be maintained with any other perfusion system, provided the residence time in the hydrocyclone is not significantly changed.

Immortal cells for the expression of tissue-specific functions *In vitro* models are important for understanding tissue functions in organs and for therapeutic applications in pharmacology and toxicology, as well as for the development of bioartificial tissue supports. Standard continuous cell lines would be suitable if it were not for the fact that they loose a substantial part of their tissue-specific properties during immortalization – they dedifferentiate like tumor cells.

Therefore, in collaboration with the Fraunhofer Institute for Toxicology and Experimental Medicine in Hanover, different immortalized hepatocytes, derived from transgenic and knockout mice, were investigated for their potential to conserve the physiological properties of primary hepatocytes under chemically defined serum-free medium conditions. The transgenic cell lines showed cytochrome-P450 activity, consumed lactate, and secreted albumin at a cell specific rate which was of the same order of magnitude found in primary hepatocytes. Moreover, the potential of detoxifying ammonium was preserved in the transgenic cells. In contrast, a tumor-like cell line derived from neonatal p53 knockout mice ceased to show any liver-specific properties. Our investigations show that immortalization of cells by transgenes can conserve tissue-type expression patterns and are suitable as continuous cell lines with specific properties.

Publications 2002

Infection and Immunity

- *Akkoyun, A. and Bilitewski, U. Optimisation of glass surfaces for optical immunosensors.* ***Biosensors and Bioelectronics****. 2002;* ***17(8)****:655-664.*
- *Arakawa, K.; Müller, R; Mahmud, T.; Yu, T.-W., and Floss, H. G. Characterization of the early stage aminoshikimate pathway in the formation of 3-amino-hydroxybenzoic acid (ahba): the rifn protein specifically converts kanosamine into kanosamine 6-phosphate.* ***Journal of the American Chemical Society****. 2002;* ***124****:10644-10655.*
- *Babaahmady, K.; Bergmeier, L. A.; Whittall, T.; Singh, M.; Wang, Y., and Lehner, T. A comparative investigation of CC chemokines and SIV suppressor factors generated by CD8+ and CD4+ T cells and CD14+monocytes.* ***Journal of Immunological Methods****. 2002;* ***264(1-2)****:1-10.*
- *Basso, H.; Rharbaoui, F.; Ständer, L. H.; Medina, E.; Garcia-Del Portillo, F., and Guzman, C. A. Characterization of a novel intracellular activated gene from* Salmonella enterica serovar typhi. ***Infection and Immunity****. 2002;* ***70****:5404-5411.*
- *Beissert, S. Use of mutant mice in photoimmunological and photocarcinogetic investigations.* ***Methods****. 2002;* ***28****:130-137.*
- *Bene, L.; Fust, G.; Huszti, Z.; Hernadi, Z.; Fekete, B.; Meszaros, M.; Veres, A.; Kovacs, A.; Miklos, K.; Singh, M.; Romics, L., and Prohaszka, Z. Impaired humoral immune response against mycobacterial 65-kDa heat shock protein (HSP65) in patients with inflammatory bowel disease.* ***Digestive Diseases and Sciences****. 2002;* ***47(7)****:1432-1437.*
- *Benesch, S.; Lommel, S.; Steffen, A.; Stradal, T. E.; Scaplehorn, N.; Way, M.; Wehland, J., and Rottner, K. PIP2-induced vesicle movement depends on N-WASP and involves Nck, WIP and Grb2.* ***Journal of Biological Chemistry****. 2002;* ***277****:37771-37776.*
- *Bierne, H.; Mazmanian, S. K.; Trost, M.; Pucciarelli, M. G. ; Dehoux, P.; the European* Listeria *Genome Consortium; Jänsch, L.; Garcia-del Portillo, F.; Schneewind, O., and Cossart, P. Inactivation of the srtA gene in* Listeria monocytogenes *inhibits anchoring of surface proteins and affects virulence.* ***Molecular Microbiology****. 2002;* ***43****:869-881.*
- *Billich, C.; Sauder, C.; Frank, R.; Herzog, S.; Bechter, K.; Takahashi, K.; Peters, H.; Staeheli, P., and Schwemmle, M. High-avidity human serum antibodies recognizing linear epitopes of borna disease virus proteins.* ***Biological Psychiatry****. 2002;* ***51(12)****:979-987.*
- *Birringer, M.; Pilawa, S., and Flohé, L. Trends in selenium biochemistry.* ***Natural Product Reports****. 2002;* ***19****:693-718.*
- *Boehme, C.; Conradt, H. S.; Nimtz, M.; Eckert, R.; Ragg, H., and Strathmann, A. Glycosylation pattern of human cofactor II from plasma and from recominant CHO cells.* ***European Journal of Biochemistry****. 2002;* ***269(3)****:977-988.*
- *Brigelius-Flohé, R. and Flohé, L. Is there a role of glutathione peroxidases in signaling and differentiation. In: (Pompella, A.; Bánhegyi, G., and Wellmam-Rousseau, M., editors). Thiol metabolism and redox regulation of cellular functions. Ohmsha: IOS Press; 2002;* ***347****:96-106. (NATO Science Series. Life and behavioural sciences. ISBN: 1 58603 282 8).*
- *Budde, H. and Flohé, L. Enzymes of the thiol-dependent hydroperoxide metabolism in pathogens as potential drug targets. In: (Pompella, A.; Bánhegyi, G., and Wellman-Rousseau, M., editors). Thiol metabolism and redox regulation of cellular functions. Ohmsha: IOS Press; 2002;* ***347****:85-95. (NATO Science Series. Life and behavioural sciences; v. I).*
- *Bächner, D.; Schröder, D., and Gross, G. mRNA expression of the murine glycoprotein (transmembrane) nmb (Gpnmb) gene is linked to the developing retinal pigment epithelium and iris.* ***Gene Expression Patterns****. 2002;* ***1****:159-165.*
- *Calmano, W.; Bilitewski, U.; Flemming, H. C.; Hofmann, T.; Peiffer, S.; Ternes, T., and Wilken, R. D. The German Water Chemical Society: Actual trends and fields of research in the principle committee „Basic Research".* ***Acta Hydrochimica et Hydrobiologica****. 2002;* ***29(6-7)****:419-427.*
- *Carvalhal, A. V.; Coroadinha, A. S.; Alves, P. M.; Moreira, J. L.; Hauser, H., and Carrondo, M. J. T. Metabolic changes during cell growth inhibition by the Irf-1 system.* ***Enzyme and Microbial Technology****. 2002;* ***30(1)****:95-109.*
- *Castro, H.; Budde, H.; Flohé, L.; Hofmann, B.; Lünsdorf, H.; Wissing, J., and Tomás, A. M. Specificity and kinetics of a mitochondrial peroxiredoxin of* Leishmania infantum. ***Free Radical Biology & Medicine****. 2002;* ***33****:1563-1574.*
- *Castro, H.; Sousa, C.; Budde, H.; Lünsdorf, H.; da Silva, A. C.; Flohé, L., and Tomás, A. M. Complementary antioxidant defence by cytoplasmic and mitochondrial peroxiredoxins in* Leishmania infantum. ***Free Radical Biology & Medicine****. 2002;* ***33****:1552-1562.*
- *Chhatwal, G. S. Anchorless adhesins and invasins of Gram-positive bacteria: a new class of virulence factors.* ***Trends in Microbiology****. 2002;* ***10(5)****:205-208.*
- *Cruz, H. J.; Conradt, H. S.; Dunker, R.; Peixoto, C. M.; Cunha, A. E.; Thomaz, M.; Burger, C.; Dias, E. M.; Clemente, J.; Moreira, J. L.; Rieke, E., and Carrondo, M. J. Process development of a recombinant antibody/interleukin-2 fusion protein expressed in protein-free medium by BHK cells.* ***Journal of Biotechnology****. 2002;* ***96(2)****:169-183.*
- *Ehlers, S.; Lehmann, J.; Müller, K.; Buer, J., and Lauber, J. Measuring immune responses. In: (Kaufmann, S. H. E., Kabelitz D. eds.) Immunology of infection, 2nd ed.,* ***Methods in Microbiology****. San Diego: Academic Press; 2002;* ***32****:403-431.*
- *Erben, R. G. ; Soegiarto, D. W.; Weber, K.; Zeitz, U.; Lieberherr, M.; Gniadecki, R.; Moller, G.; Adamski, J., and Balling, R. Deletion of deoxyribonucleic acid binding domain of the vitamin D receptor abrogates genomic and nongenomic functions of vitamin D.* ***Molecular Endocrinology****. 2002;* ***16(7)****:1524-1537.*
- *Erdogan, S.; Fagan, P. K.; Talay, S. R.; Rohde, M.; Ferrieri, P.; Flores, A. E.; Guzman, C. A.; Walker, M. J., and Chhatwal, G. S. Molecular analysis of group B protective surface protein, a new cell surface protective antigen of group B streptococci.* ***Infection and Immunity****. 2002;* ***70(2)****:803-811.*
- *Feng, X. S.; Guo, Z.; Nourbakhsh, M.; Hauser, H.; Ganster, R. W.; Shao, L. F., and Geller, D. A. The Nf-6 B repressing factor (Nrf) mediates basal repression of the human inducible nitric oxide synthase (Hinos) gene.* ***Faseb Journal****. 2002;* ***99:****14212-14217.*
- *Feng, X.; Guo, Z.; Nourbakhsh, M.; Hauser, H.; Ganster, R.; Shao, L., and Geller, D. A. Identification of a negative response element in the human inducible nitric oxide synthase (hiNOS) promoter: he role of NF-kB repressing factor (NRF) in basal repression of the hiNOS gene.* ***Proceedings of the National Academy of Sciences, U.S.A****. 2002;* ***99****:14212-14217*
- *Flohe, L.; Budde, H.; Bruns, K.; Castro, H.; Clos, J.; Hofmann, B.; Kansal-Kalavar, S.; Krumme, D.; Menge, U.; Plank-Schumacher, K.; Sztajer, H.; Wissing, J.; Wylegalla, C., and Hecht, H. J. Tryparedoxin peroxidase of* Leishmania donovani*: molecular cloning, heterologous expression, specificity, and catalytic mechanism.* ***Archives of Biochemistry and Biophysics****. 2002;* ***397(2)****:324-335.*
- *Flohe, L.; Steinert, P.; Hecht, H. J., and Hofmann, B. Tryparedoxin and tryparedoxin peroxidase.* ***Methods in Enzymology****. 2002;* ***347****:244-258.*
- *Flohé, L. ; Foresta, C.; Garolla, A.; Maiorino, M.; Roveri, A., and Ursini, F. Metamorphosis of the Selenoprotein PHGPx during Spermatogenesis.* ***Annals of the New York Academy of Sciences****. 2002;* ***973****:287-288.*

- *Foresta, C.; Flohé, L.; Garolla, A.; Roveri, A.; Ursini, F., and Maiorino, M. Male fertility is linked to the selenoprotein phospholipid hydroperoxide glutathione peroxidase.* ***Biology of Reproduction****. 2002;* ***67****:967-971.*

- *Frank, R. The SPOT-synthesis technique. Synthetic peptide arrays on membrane supports-principles and applications.* ***Journal of Immunological Methods****. 2002;* ***267(1)****:13-26.*
  Frank, R. and Balling, R. Funktionelle Genomanalyse mit synthetischen Verbindungen: das NGFN unterstützt Initiative zur Chemischen Genomik. ***GenomXPress****. 2002;* ***3/02****:20-21.*

- *Frank R. and Schneider-Mergener, J. SPOT-Synthesis - Scope of applications. In: (Koch J, Mahler M. eds.) Peptide Arrays on Membrane Supports - Synthesis and Application: Springer Verlag; 2002; pp. 1-24. (Springer lab manual).*

- *Gaitatzis, N.; Silakowski, B.; Kunze, B.; Nordsiek, G.; Blöcker, H.; Höfle, G., and Müller, R. The biosynthesis of the aromatic myxobacterial electron transport inhibitor stigmatellin is directed by a novel type of modular polyketide synthase.* ***Journal of Biological Chemistry****. 2002;* ***277(15)****:13082-13090.*

- *Geese, M.; Loureiro, J. J.; Bear, J. E.; Wehland, J.; Gertler, F. B., and Sechi, A. S. Contribution of Ena/VASP proteins to intracellular motility of* Listeria *requires phosphorylation and proline-rich core but not F-actin binding or multimerization.* ***Molecular Biology of the Cell****. 2002;* ***13(7)****:2383-2396.*

- *Gerber, J. K.; Richter, T.; Kremmer, E.; Adamski, J.; Hofler, H.; Balling, R., and Peters, H. Progressive loss of PAX9 expression correlates with increasing malignancy of dysplastic and cancerous epithelium of the human oesophagus.* ***Journal of Pathology****. 2002;* ***197(3)****:293-297.*

- *Gerth, K.; Steinmetz, H.; Höfle, G., and Reichenbach, H. Studies on the Biosynthesis of Epothilones: Hydroxylation of Epo A and B to Epothilones E and F.* ***Journal of Antibiotics****. 2002;* ***55(1)****:41-45.*

- *Gillen, C. M.; Towers, R. J.; McMillan, D. J.; Delvecchio, A.; Sriprakash, K. S.; Currie, B.; Kreikemeyer, B.; Chhatwal, G. S., and Walker, M. J. Immunological response mounted by Aboriginal Australians living in the Northern Territory of Australia against* Streptococcus pyogenes *serum opacity factor.* ***Microbiology****. 2002;* ***148(1)****: 169-178.*

- *Goldmann, O.; Rohde, M., and Medina, E. Phagocytosis of bacille Calmette-Guerin-infected necrotic macrophages induces a maturation phenotype and evokes antigen-presentation functions in dendritic cells.* ***Immunology****. 2002;* ***107(4)****:500-506.*

- *Grabbe, S. and Gunzer, M. DC-T-cell synapses.* ***Trends in Immunology****. 2002;* ***23(2)****:66.*

- *Grabenhorst, E.; Nimtz, M., and Conradt, H. S. Targeting of genetically engineered glycosyltransferases to* in vivo *functional Golgi subcompartments of mammalian cells. In: Glycosylation. Dordrecht, Boston, London: Kluwer Academic Publishers; 2002; pp. 149-170. (Al-Rubeai, M. ed.: Cell Engineering; v. 3).*

- *Gravesen, A. ; Ramnath, M.; Rechinger, K. B.; Andersen, N.; Jänsch, L.; Héchard, Y.; Hastings, J. W., and Knochel, S. High-level resistance to class IIa bacteriocins is associated with one general mechanism in* Listeria monocytogenes. ***Microbiology****. 2002;* ***148****:2361-2369.*

- *Heim, S.; Del Mar Lleo, M.; Bonato, B.; Guzman, C. A., and Canepari, P. The viable but nonculturable state and starvation are different stress responses of* Enterococcus faecalis, *as determined by proteome analysis.* ***Journal of Bacteriology****. 2002;* ***184****: 6739-6745.*

- *Henze, B.; Bebber, C.; van den Heuvel, J., and Bilitewski, U.Detection of mRNA using the BIAcore. 2002.*
  Notes: http://w210.ub.uni-tuebingen.de/dbt/volltexte/2002/454/

- *Hetzer-Egger, C.; Schorpp, M.; Haas-Assenbaum, A.; Balling, R.; Peters, H., and Boehm, T. Thymopoiesis requires Pax9 function in thymic epithelial cells.* ***European Journal of Immunology****. 2002;* ***32****(4):1175-1181.*
- *Hillmann, G. ; Steinkamp-Zucht, A.; Geurtsen, W.; Gross, G., and Hoffmann, A. Culture of primary human gingival fibroblasts on biodegradable membranes.* ***Biomaterials****. 2002;* ***23(6)****:1461-1469.*

- *Hirschmann, F.; Verhoeyen, E.; Wirth, D.; Bauwens, S.; Hauser, H., and Rudert, M. Vital marking of articular chondrocytes by retroviral infection using green fluorescence protein.* ***Osteoarthritis and Cartilage****. 2002;* ***10(2)****:109-118.*

- *Hoffmann, A. ; Czichos, S.; Kaps, C.; Bachner, D.; Mayer, H.; Kurkalli, B. G.; Zilberman, Y.; Turgeman, G.; Pelled, G.; Gross, G., and Gazit, D. The T-Box transcription factor brachyury mediates cartilage development in mesenchymal stem cell line C3h10t1/2.* ***Journal of Cell Science****. 2002;* ***115(4)****:769-781.*

- *Hofmann, B.; Hecht, H. J., and Flohe, L. Peroxiredoxins.* ***Biological Chemistry****. 2002;* ***383(3-4)****:347-364.*

- *Hollister, J.; Grabenhorst, E.; Nimtz, M.; Conradt, H. S., and Jarvis, D. L. Engineering of the protein N-glycosylation pathway in insect cells for the production of biantennary complex N-glycans.* ***Biochemistry-US****. 2002;* ***41(50)****:15093-15104.*

- *Hollnagel, A.; Grund, C.; Franke, W. W., and Arnold, H. H. The cell adhesion molecule M-cadherin is not essential for muscle development and regeneration.* ***Molecular and Cellular Biology****. 2002;* ***13****:4760.*

- *Holtkötter, O.; Nieswandt, B.; Smyth, N.; Müller, W.; Hafner, M.; Schulte, V.; Krieg, T., and Eckes, B. Integrin alpha 2-deficient mice develop normally, are fertile, but display partially defective platelet interaction with collagen.* ***Journal of Biological Chemistry****. 2002;* ***277(13)****:10789-94.*

- *Horvath, L.; Cervenak, L.; Oroszlan, M.; Prohaszka, Z.; Uray, K.; Hudecz, F.; Baranyi, E.; Madacsy, L.; Singh, M.; Romics, L.; Fust, G., and Panczel, P. Antibodies against different epitopes of heat-shock protein 60 in children with type 1* Diabetes mellitus. ***Immunology Letters****. 2002;* ***80(3)****:155-62.*

- *Jansen, R.; Kunze, B.; Reichenbach, H., and Höfle, G. The Ajudazols a and B, Novel isochromanones from* Chondromyces crocatus *(Myxobacteria): Isolation and structure elucidation.* ***European Journal of Organic Chemistry****. 2002;* ***(5)****:917-921.*

- *Jansen, W. T. M.; Bolm, M.; Balling, R.; Chhatwal, G. S., and Schnabel, R. Hydrogen peroxide-mediated killing of* Caenorhabditis elegans *by* Streptococcus pyogenes. ***Infection and Immunology****. 2002;* ***70****:5202-5207.*

- *Jenke, B. H. C.; Fetzer, C. P.; Stehle, I. M.; Jonsson, F.; Fackelmayer, F. O.; Conradt, H.; Bode, J., and Lipps, H. J. An episomally replicating vector binds to the nuclear matrix protein Saf-a in vivo.* ***EMBO Reports****. 2002;* ***3(4)****:349-354.*

- *Jorgensen, C.; Noel, D., and Gross, G. Could inflammatory arthritis be triggered by progenitor cells in the joints?* ***Annals of the Rheumatic Diseases****. 2002;* ***61(1)****:6-9.*

- *Kalabay, L.; Fekete, B.; Czirjak, L.; Horvath, L; Daha, M. R.; Veres, A.; Fonyad, G.; Horvath, A.; Viczian, A.; Singh, M.; Hoffer, I.; Fust, G.; Romics, L., and Prohaszka, Z.* Helicobacter pylori *infection in connective tissue disorders is associated with high levels of antibodies to mycobacterial hsp65 but not to human hsp60.* ***Heliobacter****. 2002;* ***7(4)****:250-6.*

- *Kaps, C.; Bramlage, C.; Smolian, H.; Haisch, A.; Ungethüm, U.; Burmester, G. R.; Sittinger, M.; Gross, G., and Haupl, T. Bone morphogenetic proteins promote cartilage differentiation and protect engineered artificial cartilage from fibroblast invasion and destruction.* ***Arthritis and Rheumatism****. 2002;* ***46(1)****:149-162.*

- *Kärst, U. Realis: Postgenomic Analysis of* Listeria monocytogenes. ***Comparative and Functional Genomics****. 2002;* ***3(1)****:32-34.*

- *Katz, E.; Ichia, L. S. H.; Bückmann, A. F., and Willner, I. Dual biosensing by magneto-controlled bioelectrocatalysis.* ***Angewandte Chemie****. 2002;* ***114****:1399-1402.*

- *Kellersmann, R.; Lazarovits, A.; Grant, D.; Garcia, B.; Chan, B.; Kellersmann, A.; Wang, H.; Jevnikar, A.; Wagner, N.; Müller, W.; Ulrichs, K.; Thiede, A., and Zhong, R. Monoclonal antibody against beta 7 integrins, but not beta 7 deficiency, attenuates intestinal allograft rejection in mice.* ***Transplantation****. 2002;* ***74****:1327-1334.*

- *Kim, T.-Y.; Vargas, V.; Mayer, H.; Somjen, D., and Kaye, A. M. Selective anabolic effects of muteins of mid-region PTH fragments on skeletal tissues of prepubertal rats.* ***Bone****. 2002;* ***30****:78-84.*

- *Kreikemeyer, B.; Chhatwal, G. S., and Walker, M. J. Immunological response mounted by Aboriginal Australians living in the Northern Territory of Australia against* Streptococcus pyogenes *serum opacity factor.* ***Microbiology****. 2002;* ***148****:167-178.*

- *Kresse, A. U.; Ebel, F., and Guzman, C. A., authors. Functional modulation of pathogenic bacteria upon contact with host target cells. In: (Wilson, M., editor). Advances in molecular and cellular microbiology Bacterial adhesion to host tissues: mechanisms and consequences. Cambridge, UK: Cambridge University Press; 2002; Chapter* ***9****:203-220.*

- *Kretschmer, K.; Engel, H., and Weiss, S. Strong antigenic selection shaping the immunoglobulin heavy chain repertoire of B1a- lymphocytes in* $\square^{315}$ *transgenic mice.* ***European Journal of Immunology****. 2002; 32* ***(8)****:2317-2327.*

- *Kröger, A.; Köster, M.; Schroeder, K.; Hauser, H., and Müller, P. P. Activities of IRF-1.* ***Journal of Interferon and Cytokine Research****. 2002;* ***22(1)****:5-14.*

- *Krumme, D.; Hecht, H. J.; Menge, U.; Ross, A.; Wray, V., and Flohe, L. 1H, 15N and 13C resonance assignments and secondary structure of tryparedoxin-I from* Crithidia fasciculata. ***Journal of Biomolecular*** *NMR. 2002;* ***22(4)****:375-376.*

- *Krusch, S.; Domann, E.; Frings, M.; Zelmer, A.; Diener, M.; Chakaborty, T., and Weiss, S.* Listeria monocytogenes *mediated CFTR transgene transfer to mammalian cells.* ***The Journal of Gene Medicine****. 2002;* ***4(6)****:655-667.*

- *Kusnick, C.; Jansen, R.; Liberra, K., and Lindequist, U. Ascochital, a new metabolite from the marine ascomycete* Kirschsteiniothelia maritima. ***Pharmazie****. 2002;* ***57(7)****:510-512.*

- *Lechner, O.; Bruder, D.; Lauber, J., and Buer, J. Anerge T-Zellen: Immunregulatoren mit Potential für die Klinik!* ***Die Gelben Hefte****. 2002;* ***42****:17-26.*

- *Lechner, O.; Bruder, D.; Lauber, J., and Buer, J. Anergic T-cells: immunregulators with clinical implications!* ***Biomedical Progress****. 2002;* ***15****:11-16.*

- *Liebich, I.; Bode, J.; Frisch, M., and Wingender, E. S/MARt DB: a database on scaffold/matrix attached regions.* ***Nucleic Acids Research****. 2002;* ***30(1)****:372-374.*

- *Liebich, I.; Bode, J.; Reuter, I., and Wingender, E. Evaluation of sequence motifs found in scaffold/matrix-attached regions (S/MARs).* ***Nucleic Acids Research****. 2002;* ***30(15)****:3433-3442.*

- *Lindroth, K.; Troye-Blomberg, M.; Singh, M.; Dieli, F.; Ivanyi, J., and Fernandez, C. The humoral response in TCR alpha-/- mice. Can gammadelta-T cells support the humoral immune response?* ***Scandinavian Journal of Immunology****. 2002;* ***55(3)****:256-263.*

- *Lührmann, A.; Deiters, U.; Skokowa, J.; Hanke, M.; Gessner, J. E.; Mü hlradt, P. F.; Pabst, R., and Tschernig, T. In vivo effects of a synthetic 2-kilodalton macrophage-activating lipopeptide of* Mycoplasma fermentans *after pulmonary application.* ***Infection and Immunity****. 2002;* ***70(7)****:3785-3792.*

- *Mahmud, T.; Bode, H. B.; Silakowski, B.; Kroppenstedt, R. M.; Xu, M.; Nordhoff, S.; Höfle, G., and MüllerR. A novel biosynthetic pathway providing precursors for fatty acid biosynthesis and secondary metabolite formation in Myxobacteria.* ***Journal of Biological Chemistry****. 2002;* ***277****:32768-32774.*

- *Medina, E.; Anders, D., and Chhatwal, G. S. Induction of NF-6B nuclear translocation in human respiratory epithelial cells by group A streptococci.* ***Microbial Pathogenesis****. 2002;* ***33(6)****:307-313.*

- *Medina, E. and Chhatwal, G. S. The potential for vaccine development against rheumatic fever.* ***Indian Heart Journal****. 2002;* ***54(1)****:93-98.*

- *Meraro, D.; Gleit-Kielmanowicz, M.; Hauser, H., and Levi, B. Z. IFN-stimulated gene 15 Is synergistically activated through interactions between the myelocyte/lymphocyte-specific transcription factors, PU.1, IFN regulatory factor-8/IFN consensus sequence binding protein, and IFN regulatory factor-4: characterization of a new subtype of IFN-stimulated response element.* ***Journal of Immunology****. 2002;* ***168(12)****:6224-31.*

- *Mielke, C.; Benham, C.; Bode, J., and Breindl, M. Nuclear matrix association potential of regulatory elements in the collagen 1a1 gene.* ***Journal of Cellular Biochemistry****. 2002;* ***84****:DOI 10.1002/jcb.10034.*

- *Mielke, C.; Christensen, M. O.; Westergaard, O.; Bode, J.; Benham, C. J., and Breindl, M. Multiple collagen I gene regulatory elements have sites of stress- induced DNA duplex destabilization and nuclear scaffold/matrix association potential.* ***Journal of Cellular Biochemistry****. 2002;* ***84(3)****:484-496.*

- *Morr, M.; Takeuchi, O.; Akira, S.; Simon, M., and Mühlradt, P. F. Differential recognition of structural details of bacterial lipopeptides by toll-like receptors.* ***European Journal of Immunology****. 2002;* ***32****:3337-3347.*

- *Munder, A.; Krusch, S.; Tschernig, T.; Dorsch, M.; Lührmann, A.; van Griensven, M.; Tümmler, B.; Weiß, S., and Hedrich, H.-J. Pulmonary microbial infection in mice: comparison of different application methods and correlation of bacterial numbers and histopathology.* ***Experimental and Toxicologic Pathology****. 2002;* ***54(2)****:127-133.*

- *MüllerK. and Wirth, M. Real-time RT-PCR detection of retroviral contaminations of cells and cell lines.* ***Cytotechnology****. 2002;* ***38(1)****:147-153.*

- *Niggemann, J.; Frank, R.; Michaelis, K.; Zander, N., and Höfle, G. Natural product-derived building blocks for combinatorial synthesis: structural diversity by fragmentation and recombination of natural products from Myxobacteria.* ***Journal of Chemical Society, Perkin Transactions****. 2002;* ***1****:2490-2503.*

- *Olazabal, I.; Caron, E.; May, R.; Schilling, K.; Knecht, D., and Machesky, L. Rho-kinase and myosin-II control phagocytic cup formation during CR, but not FcgammaR, phagocytosis.* ***Current Biology****. 2002;* ***12(16)****:1413.*

- *Pägelow, U.; Wirth, M.; Buhr, P.; Macke, L.; Hannig, H.; Dittmar, K. E. J.; Berlin, J.; Wörmann, B., and Lindenmaier, W. Adenovirally modified dendritic cells for immunotherapy: from basic development to clinical application. Dordrecht, Boston, London: Kluwer Academic Press; 2002; pp. 510-516. (Lindner-Olssoon, E. Chatzissavidou N. Lüllau E., eds.)*

- *Pasparakis, M.; Courtois, G.; Hafner, M.; Schmidt-Supprian, M.; Nenci, A.; Toksoy, A.; Kramfpert, M.; Goebeler, M.; Gillitzer, R.; Israel, A.; Krieg, T.; Rajewski, K., and Haase, I. TNF-mediated inflammatory skin disease in mice with epidermis-specific ablation of IKK2.* ***Nature****. 2002;* ***417****:861-866.*

- *Pradella, S. ; Hans, A.; Spröer, C.; Reichenbach, H.; Gerth, K., and Beyer, S. Characterisation, genome size, and genetic manipulation of* Sorangium cellulosum *So ce56.* ***Archives of Microbiology****. 2002;* ***178(6)****:484-492.*

- *Prohaszka, Z.; Singh, M.; Nagy, K.; Kiss, E.; Lakos, G.; Duba, J., and Fust, G. Heat shock protein 70 is a potent activator of the human complement system.* ***Cell Stress Chaperones****. 2002;* ***7(1)****: 17-22.*

- *Pukall, R.; Kramer, I.; Rohde, M., and Stackebrandt, E. Microbial diversity of cultivatable bacteria associated with the north seo bryozoan* Flustra foliciae. ***Systems of Applied Microbiology****. 2002;* ***24****: 623-633.*

- *Rasmussen, U. B.; Schreiber, V.; Schultz, H.; Mischler, F., and Schughart, K. Tumor cell-targeting by Phage-displayed peptides.* ***Cancer Gene Therapy****. 2002;* ***9(7)****:606-612.*

- *Regueiro-Ren, A.; Leavitt, K.; Kim, S.-H.; Höfle, G.; Gougoutas, J.; DiMarco, J.; Lee, F. Y. F.; Fairchild, C. R.; Long, B. H., and Vite, G. D. SAR and pH stability of cyano-substitued epothilones.* ***Organic Letters****. 2002;* ***4(22)****:3815-3818.*

- *D. J. Reinscheid, C. Stosser, K. Ehlert, R. W. Jack, K. Moller, B. J. Eikmanns, G. S. Chhatwal Influence of proteins Bsp and FemH on cell shape and peptidoglycan composing in group B* streptococcus. ***Microbiologie****. 2002;* ***148****:3245-54*

- *Rharbaoui, F.; Drabner, B.; Borsutzky, S.; Winckler, U.; Morr, M.; Ensoli, B.; Mühlradt, P. F., and Guzman, C. A. The* Mycoplasma*-derived lipopeptide MALP-2 is a potent mucosal adjuvant.* ***European Journal of Immunology****. 2002;* ***32****:2857-2865.*

- *Richter, T.; Shultz-Lockyear, L. L.; Oleschuk, R. D.; Bilitewski, U., and Harrison, D. J. Bi-enzymatic and capillary electrophoretic analysis of non- fluorescent compounds in microfluidic devices - determination of xanthine.* ***Sensors and Actuators B-Chemical****. 2002;* ***81(2-3)****:369-376.*

- *Rohde, M.; Schwienbacher, M.; Nikolaus, T.; Heesemann, J., and Ebel, F. Detection of early phase specific surface appendages during germination of* Aspergillus fumigatus. ***FEMS Microbiology Letters****. 2002;* ***206****:99-105.*

- *Romanenko, L. A.; Schuhmann, P.; Rohde, M.; Mikhailov, V. V., and Stackebrandt, E.* Halomonas halocynthiae *sp. nov., isolated from the marine ascidian* Halocynthia aurantium. ***International Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52****:1767-1772.*

- *Romanenko, L. A.; Schumann, P.; Rohde, M.; Lysenko, A. M. ; Mikhailov, V. V., and Stackebrandt, E.* Psychrobacter submarinus *sp. nov. and* Psychrobacter marincola *sp. nov., psychrophilic halophiles from marine environments.* ***Internatioal Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52(4)****:1291-1297.*

- *Römling, U. Molecular biology of cellulose production in bacteria.* ***Research in Microbiology****. 2002;* ***153****:205-212.*

- *Roveri, A.; Flohé, L.; Maiorino, M., and Ursini, F. Phospholipid hydroperoxide glutathione peroxidase in sperm.* ***Methods in Enzymology****. 2002;* ***347****:208-212.*

- *Ruzgas, T.; Lindgren, A.; Gorton, L.; Hecht, H.-J.; Reichelt, J., and Bilitewski, U., Electrochemistry of peroxidases. In: (Bajter-Toth, A. and Chambers, J. Q., editors). Electroanalytical methods of biological materials. Marcel Dekker Inc.; 2002; pp. 233-254.*

- *Sagi, D.; Peter-Katalinic, J.; Conradt, H. S., and Nimtz, M. Sequencing of tri- and tetraantennary N-glycans containing sialic acid by negative mode ESI QTOF tandem MS.* ***Journal of.the American Society for Mass Spectrometry****. 2002;* ***13(9)****:1138-1148.*

- *Salo, H.; Aitio, O.; Ilves, K.; Bencomo, E.; Toivonen, S.; Penttila, L.; Niemela, R.; Salminen, H.; Grabenhorst, E.; Renkonen, R., and Renkonen, O. Several polylactosamine-modifying glycosyltransferases also use internal GalNAcbeta1-4GIcNAc units of synthetic saccharides as acceptors.* ***Glycobiology****. 2002;* ***12(3)****:217-218.*

- *Sasse, F.; Steinmetz, H.; Schupp, T.; Petersen, F.; Memmert, K.; Hofmann, H.; Heusser, C.; Brinkmann, V.; Von Matt, P.; Hofle, G., and Reichenbach, H. Argyrins, immunosuppressive cyclic peptides from myxobacteria - I. Production, isolation, physico-chemical and biological properties.* ***Journal of Antibiotics****. 2002;* ***55(6)****:543-551.*

- *Schmidt, A. ; Schumacher, J. T.; Reichelt, J.; Hecht, H. J., and Bilitewski, U. Mechanistic and molecular investigations on stabilization of horseradish peroxidase C.* ***Analytical Chemistry****. 2002;* ***74(13)****:3037-3045.*

- *Schrader, A. J.; Lechner, O.; Templin, M.; Dittmar, K. E.; Machtens, S.; Mengel, M.; Probst-Kepper, M.; Franzke, A.; Wollensak, T.; Gatzlaff, P.; Atzpodien, J.; Buer, J., and Lauber, J. CXCR4/CXCL12 expression and signalling in kidney cancer.* ***British Journal of Cancer****. 2002;* ***86(8)****:1250-1256.*

- *Schroeder, K.; Koschmieder, S.; Ottmann, O. G.; Hoelzer, D.; Hauser, H., and Müller, P. P. Coordination of cell growth in cocultures by a genetic proliferation control system.* ***Biotechnology and Bioengineering****. 2002;* ***78(3)****:346-352.*

- *Schubert, A.; Zakikhany, K.; Schreiner, M.; Frank, R. ; Spellerberg, B.; Eikmanns, B. J., and Renscheid, D. J. A fibrinogen receptor from group B Streptococcus interacts with fibrinogen by repetitive units with novel ligand-binding sites.* ***Molecular Microbiology****. 2002;* ***2****:557-569.*

- *Schubert, W. D.; Moser, J.; Heinz, D. W., and Jahn, D. Structure and function of glutamyl-tRNA reductases, the first enzyme of tetrapyrrole biosynthesis in plants and bacteria.* ***Photosynthesis Research****. 2002;* ***74****:205-215.*

- *Schubert, W.-D.; Urbanke, C.; Ziehm, T.; Beier, V.; Machner, M. P.; Domann, E.; Wehland, J.; Chakraborty, T., and Heinz, D. W. Structure of the complex of internalin, a major invasion protein of* Listeria monocytogenes *with its human receptor, E-cadherin.* ***Cell****. 2002;* ***111****:825-836.*

- *Schughart, K. and Rasmussen, U. B. Solvoplex synthetic vector for intrapulmonary gene delivery. Preparation and use.* ***Methods in Molecular Medicine****. 2002;* ***69****:83-94.*

- *Schultz, A. ; Laschat S.; Morr, M.; Diele, S.; Dreyer, M., and Bringmann, G. Highly branched alkanoic acids from the preen-gland wax of the domestic goose as building blocks for chiral triphenylenes.* ***Helvetica Chimica Acta****. 2002;* ***85****:3909-3018.*

- *Sechi, A. S.; Buer, J.; Wehland, J., and Probst-Kepper, M. Changes in actin dynamics at the T-cell/APC interface: implications for T-cell anergy?* ***Immunological Reviews****. 2002;* ***189****:98-110.*

- *Siggelkow, H.; Schenck, M.; Rohde, M.; Viereck, V.; Tauber, S., and Hüfner, M. Prolonged culture of HOS 58 osteosarcoma cells with 1,35-(OH)2-D3, TGF beta and dexamethason reveal physiological regulation of alkine phosphatase but compromised osteocalcin gene expression and protein synthesis and lack of minaralization.* ***Journal of Cellular Biochemistry****. 2002;* ***85****:279-294.*

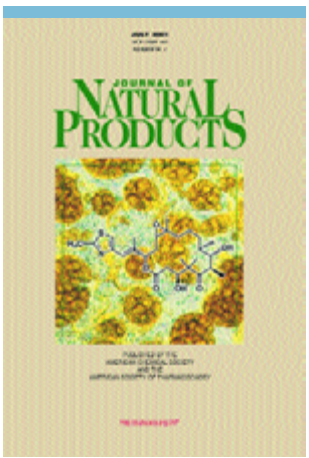
- *Small, J. V.; Stradal, T.; Vignal, E., and Rottner, K. The lamellipodium :Where motility begins.* ***Trends in Cell Biology****. 2002;* ***12****:112-120.*

- *Stander, S.; Gunzer, M.; Metze, D.; Luger, T., and Steinhoff, M. Localization of micro-opioid receptor 1A on sensory nerve fibers in human skin.* ***Regulatory Peptides****. 2002;* ***110(1)****:75-83.*

- *Stiene, M. and Bilitewski, U. Electrochemical characterization of screen-printed carbonaceous electrodes for the determination of peroxidase activity in novel screen- printed flow-through modules.* ***Analytical and Bioanalytical Chemistry****. 2002;* ***372(2)****:240-247.*

- *Sun, X.; Qiao, H.; Shi, J.; Kanwar, J. R.; Müller, W.; Wagner, N., and Krissansen, G. W. Beta7 integrins contribute to skin graft rejection.* ***Transplantation****. 2002;* ***74****:1202-1203.*

- *Sydora, B. C.; Wagner, N.; Lohler, J.; Yakoub, G.; Kronenberg, M.; Müller, W., and Aranda, R. Beta 7 integrin expression is not required for the localization of T cells to the intestine and colitis pathogenesis.* ***Clinical and Experimental Immunology****. 2002;* ***129(1)****:35-42.*
- *Tiedemann v., B. and Bilitewski, U. Characterization of the vascular endothelial growth factor - receptor interaction and determination of the recombinant protein by an optical receptor sensor.* ***Biosensors and Bioelectronics****. 2002;* ***17****:983-991.*
- *Toi, M.; Bando, H.; Ogawa, T.; Muta, M.; Hornig, C., and Weich, H. A. Significance of vascular endothelial growth factor (Vegf)/ Soluble Vegf Receptor-1 relationship in breast cancer.* ***International Journal of Cancer****. 2002;* ***98(1)****:14-18.*
- *Trobonjaca, Z.; Kröger, A.; Stober, D.; Leithauser, F.; Moller, P.; Hauser, H.; Schirmbeck, R., and Reimann, J. Activating immunity in the liver. II. IFN-beta attenuates NK cell- dependent liver injury triggered by liver NKT cell activation.* ***Journal of Immunology****. 2002;* ***168(8)****:3763-3770.*
- *Van den Heuvel, J. and Heinz, D. W. „Plug and Play"-expression systems for high-quality production of recombinant proteins for structural analysis.* ***Gene Function and Diseases****. 2002;* ***3****:33-38.*
- *Van Griensven, M.; Lobenhoffer, P.; Barke, A.; Tschernig, T.; Lindenmaier, W.; Krettek, C., and Gerich, T. G. Adenoviral gene transfer in a rat fracture model.* ***Laboratory Animals****. 2002;* ***36****:455-461.*
- *Veres, A.; Fust, G.; Smieja, M.; McQueen, M.; Horvath, A.; Yi, Q.; Biro, A.; Pogue, J.; Romics, L.; Karadi, I.; Singh, M.; Gnarpe, J.; Prohaszka, Z., and Yusuf, S. Relationship of anti-60 kDa heat shock protein and anti-cholesterol antibodies to cardiovascular events.* ***Circulation****. 2002;* ***106(22)****:2775-80.*
- *Veres, A.; Prohászka, Z.; Kilpinen, S.; Singh, M.; Füst, G., and Hurme, M. The promoter polymorphism of the IL-6 gene is associated with levels of antibodies to 60-kDa heat-shock proteins.* ***Immunogenetics****. 2002;* ***53(10-11)****:851-856.*
- *Veres, A.; Szamosi, T; Ablonczy, M.; Szamosi, T. Jr.; Singh, M.; Karadi, I.; Romics, L.; Fust, G., and Prohaszka, Z. Complement activating antibodies against the human 60 kDa heat shock protein as a new independent family risk factor of coronary heart disease.* ***European Journal of Clinical Investigation****. 2002;* ***32(6)****:405-10.*
- *Vollbrecht, L.; Steinmetz, H.; Höfle, G.; Oberer, L.; Rhis, G.; Bovermann, G., and Von Matt, P. Argyrins, immunosuppressive cyclic peptides from Myxobacteria - II. Structure elucidation and stereochemistry.* ***Journal of Antibiotics****. 2002;* ***55****:715-721.*
- *Von Minden, H. M.; Morr, M.; Milkereit, G.; Heinz, E., and Vill, V. Synthesis and mesogenic properties of glycosyl diacilglycerols.* ***Chemistry and Physics of Lipids****. 2002;* ***114****:55-80.*
- *Wagner, A.; Ekhlasi-Hundrieser, M.; Hettel, C.; Petrunkina, A.; Waberski, D.; Nimtz, M., and Topfer-Petersen, E. Carbohydrate-based interactions of oviductal sperm reservoir formation-studies in the pig.* ***Molecular Reproduction and Development****. 2002;* ***61(2)****:249-57.*
- *Wang, Y.; Kelly, C. G.; Singh, M.; McGowan, E. G.; Carrara, A. S.; Bergmeier, L. A., and Lehner, T. Stimulation of Th1-polarizing cytokines, C-C chemokines, maturation of dendritic cells, and adjuvant function by the peptide binding fragment of heat shock protein 70.* ***Journal of Immunology****. 2002;* ***169(5)****:2422-9.*
- *Wilde, C.; Chhatwal, G. S., and Aktories, K. C3stau, a new member of the family of C3-like ADP-ribosyltransferases.* ***Trends in Microbiology****. 2002;* ***10(1)****:5-7.*
- *Winkler, J.; Hagelstein, S.; Rohde, M., and Laqua, H. Cellular and cytoskeletal dynamics within organ cultures of porcine neuroretina.* ***Experimental Eye Research****. 2002;* ***74****:777-788.*
- *Winterhoff, N.; Goethe, R.; Gruening, P.; Rohde, M.; Kalisz, H.; Smith, H. E., and Valentin-Weigand, P. Identification and charakterization of two stress-induced surface-associated proteins of* Streptococcus suis *with high homologies to members of the arginine deiminase system of* Streptococcus pyogenes. ***Journal of Bacteriology****. 2002;* ***184****:6768-6776.*
- *Witmer, A. N.; Blaauwgeers, H. G.; Weich, H. A.; Alitalo, K.; Vrensen, G. F., and Schlingemann, R. O. Altered expression patterns of Vegf receptors in human diabetic retina and in experimental Vegf-induced retinopathy in monkey.* ***Investigative Ophthalmology and Visual Science****. 2002;* ***43(3)****:849-857.*
- *Witmer, A. N.; Dai, J. P.; Weich, H. A.; Vrensen, G. F., and Schlingemann, R. O. Expression of vascular endothelial growth factor receptors 1, 2, and 3 in quiescent endothelia.* ***Journal of Histochemistry and Cytochemistry****. 2002;* ***50(6)****:767-777.*
- *Zähner, D.; Kaminski K.; van der Linden, M.; Mascher, T.; Merai, M., and Hakenbeck, R. The ciaR/ciaH regulatory network of* Streptococcus pneumoniae. ***Journal of Molecular Microbiology and Biotechnology****. 2002;* ***4****:211-216.*
- *Zander, N. and Gausepohl, H., authors. Chemistry of Fmoc peptide synthesis on membranes. In: (Koch, J. and Mahler, M., editors). Peptide arrays on membrane supports - synthesis and applications: Springer lab manual. Berlin: Springer-Verlag; 2002; pp. 23-40.*



- *Hardt, I. H.; Steinmetz, H.; Gerth, K.; Sasse, F.; Reichenbach, H., and Höfle, G. New natural epothilones from* Sorangium cellulosum, *strains So ce90/B2 and So ce90/D13: isolation, structure elucidation, and SAR studies.* ***Journal of Natural Products****, 2001;* ***64(7)****:847-856. This paper was given the Arthur E. Schwarting Award by the American Society of Pharmacognosy for the "Best Paper 2001" in the Journal of Natural Products. The permission of the American Chemical Society is gratefully acknowledged.*

Comparative Genome Research

- *Bi, J. X.; Wirth, M.; Beer, C.; Kim, E. J.; Gu, M. B., and Zeng, A. P. Dynamic characterization of recombinant Chinese hamster ovary cells containing an inducible c-fos promoter GFP expression system as a biomarker.* ***Journal of Biotechnology****. 2002;* ***93(3)****:231-242.*
- *Chevrier, V.; Piel; Collomb, N.; Saoudi, Y.; Frank, R.; Paintrand, M.; Narumiya, S.; Bornens, M., and Job, D. The Rho-associated protein kinase p160ROCK is required for centrosome positioning.* ***Journal of Cellular Biology****. 2002;* ***157(5)****:807-817.*
- *Dostmann, W. R. G.; Tegge, W.; Frank, R.; Nickl, C. K.; Taylor, M. S., and Brayden, J. E. Exploring them mechanisms of vascular smooth muscle tone with highly specific, membrane-permeable inhibitors of cyclic GMP-dependent protein kinase I".* ***Pharmacology and Therapeutics****. 2002;* ***93(2-3)****:203-215.*
- *Eichler, J. and Houghten, R. A. Combinatorial synthesis. Goodman, M.; Felix, A.; Moroder, L., and Toniolo, C. Synthesis of peptides. Stuttgart: Thieme; 2002; chapter 4.3.7.*

- *Ernest, S.; Christensen, B.; Gilfix, B. M.; Mamer, O. A.; Hosack, A.; Rodier, M.; Colmenares, C.; McGrath, J.; Bale, A.; Balling, R.; Sankoff, D.; Rosenblatt, D. S., and Nadeau, J. H. Genetic and molecular control of folate-homocysteine metabolism in mutant mice.* ***Mammalian Genome****. 2002;* ***13(5)****:259-267.*
- *Frank, R. Chemische Genomik: funktionelle Genomanalyse mit synthetischen Verbindungen.* ***BioSpektrum****, Sonderausgabe „Proteomics and Drug Development". 2002; 474-477.*
- *Frank, R. High-density synthetic peptide microarrays: emerging tools for fuctional genomics and proteomics.* ***Combinatorial Chemistry and High Throughput Screening****. 2002;* ***5****:327-335.*
- *Frank, R. and Balling, R. Funktionelle Genomanalyse mit synthetischen Verbindungen: das NGFN unterstützt Initiative zur Chemischen Genomik.* ***GenomXPress****. 2002;* ***3/02****:20-21.*
- *Frank, R.; Bialek, K., and Swistowski, A. Large-scale protein-interaction mapping with synthetic peptide arrays: epitope-targeted proteome analysis. Peptides 2002; Proceedings 27th European Peptide Symposium, pp. 102-103.*
- *Frere, F.; Schubert, W.-D.; Neier, R., and Jahn, D. Heinz D. W. Structure of porphobilinogen synthase from* Pseudomonas aeruginosa *in complex with 5-fluorolevulinic acid suggests a double Schiff base mechanism.* ***Journal of Molecular Biology****. 2002;* ***320****:237-247.*
- *Frisch, M.; Frech, K.; Klingenhoff, A. Cartharius, K.; Liebich, I., and Werner, T.* In silico *prediction of scaffold/matrix attachment regions in large genomic sequences.* ***Genome Research****. 2002;* ***12(2)****:349-54.*
- *Hartlep, M.; Hussmann, W.; Prayitno, N.; Maynial-Salles, I., and Zeng, A.-P. Study of two-stage processes for the microbial production of 1,3-propanediol from glucose.* ***Applied Microbiology and Biotechnology****. 2002;* ***60****:60-66.*
- *Heinz, D. W. Modellsystem für Infektionen - Pathogene Bakterien auf ihrem unheilvollen Weg verfolgt.* ***Jahreshefte der Helmholtz-Gesellschaft****. 2002; 10-11.*
- *Heinz, D. W. and Jahn, D. Strukturbiologie zur gerichteten Wirkstoffentwicklung.* ***CHEManager****. 2002;* ***11****:14.*
- *Kel-Margoulis, O. V.; Ivanova, T. G.; Wingender, E., and Kel, A. E. Automatic annotation of genomic regulatory sequences by searching for composite clusters.* ***Pacific Symposium on Biocomputing****. 2002;* ***7****:187-98.*
- *Kel-Margoulis, O. V.; Kel, A. E.; Reuter, I.; Deineko, I. V., and Wingender, E. TRANSCompel®: a database on composite regulatory elements in eukaryotic genes.* ***Nucleic Acids Research****. 2002;* ***30(1)****:332-334.*
- *Kist, R.; Schrewe, H.; Balling, R., and Scherer, G. Conditional inactivation of Sox9: a mouse model for campomelic dysplasia.* ***Genesis****. 2002;* ***32(2)****:121-123.*
- *Kloos, D. U.; Choi, C., and Wingender, E. The TGF-beta--Smad network: introducing bioinformatic tools.* ***Trends in Genetics****. 2002;* ***18(2)****:96-103.*
- *Liebich, I.; Bode, J.; Frisch, M., and Wingender, E. S/MARt DB: a database on scaffold/matrix attached regions.* ***Nucleic Acids Research****. 2002;* ***30(1)****:372-374.*
- *Liebich, I.; Bode, J.; Reuter, I., and Wingender, E. Evaluation of sequence motifs found in scaffold/matrix-attached regions (S/MARs).* ***Nucleic Acids Research****. 2002;* ***30(15)****:3433-42.*
- *Ma, Z. Y.; Yuan, Y. J.; Wu, J. C., and Zeng, A. P. Apoptotic cell death in suspension cultures of* Taxus chinensis *var.* mairei*.* ***Biotechnology Letters****. 2002;* ***24(7)****:573-577.*
- *Modak, J.; Deckwer, W.-D., and Zeng, A.-P. Metabolic control analysis of eucaryotic pyruvate dehydrogenase multienzyme-complex.* ***Biotechnology Progress****. 2002;* ***18****:1157-1169.*
- *Moser, J.; Schuber, W.-D.; Heinz, D. W., and Jahn, D. Structure and function of glutamyl-tRNA-reductase involved in 5-aminolevulinic acid formation.* ***Biochemical Society of Transactions****. 2002;* ***30****:579-584.*
- *Potapov, A. P. and Wingender, E. Representing the architecture of signal tansduction networks in an algebraic form: protein target finding.* ***Annals of the NewYork Academy of Sciences****. 2002;* ***973****:1-2.*
- *Sabra, W.; Kim, E. J., and Zeng, A.-P. Physiological responses of* Pseudomonas aeruginosa *PAO1 to oxidative stress in controlled microaerobic an aerobic culture.* ***Microbiology****. 2002;* ***148****:3195-3202.*
- *Schauer, S.; Chaturvedi, S.; Randau, L.; Moser, J.; Kitabatake, M.; Lorenz, S.; Verkamp, E.; Schubert, W.-D.; Nakayashiki, T.; Murai, M.; Wall, K.; Thomann, U.; Heinz, D. W.; Inokuchi, H.; Söll, D., and Jahn, D.* Escherichia coli *glutamyl-tRNA reductase: Trapping the thioester intermediate.* ***Journal of Biological Chemistry****. 2002;* ***247(50)****:48656-48663.*
- *Schubert, W. D.; Moser, J.; Heinz, D. W., and Jahn, D. Structure and function of glutamyl-tRNA reductases, the first enzyme of tetrapyrrole biosynthesis in plants and bacteria.* ***Photosynthesis Research****. 2002;* ***74****:205-215.*
- *Schulz, H.; Johner, C.; Eder, G.; Ziesenis, A.; Reitmeier, P.; Heyder, J., and Balling, R. Respiratory mechanics in mice: strain and sex specific differences.* ***Acta Physiologica Scandinavica****. 2002;* ***174(4)****:367-375.*
- *Schügerl, K. and Zeng, A.-P., authors. Tools and applications of biochemical engineering science. Advances in Biochemical Engineering/Biotechnology. Heidelberg: Springer Verlag; 2002; 74.*
- *Vreugde, S.; Ereven, A.; Kros, C. J.; Marcotti, W.; Fuchs, H.; Kurima, K.; Wilcox, E. R.; Friedman, T. B.; Griffith, A. J.; Balling, R.; De Angelis, M. H.; Avraham, K. B., and Steel, K. P. Beethoven, a mouse model for dominant, progressive hearing loss Dfna36.* ***Nature Genetics****. 2002;* ***30(3)****:257-258.*
- *Wingender, E. Modeling regulatory pathways with the use of the TRANSFAC system.* ***Gene Function and Disease****. 2002;* ***3****:3-11.*
- *Xiu, Z.-L.; Chang, Z.-Y., and Zeng, A.-P. Nonlinear dynamics of regulation of bacterial trp operon: model analysis of integrated effects of repression, feedback inhibition and attenuation.* ***Biotechnology Progress****. 2002;* ***18****:686-693.*
- *Xiu, Z.-L.; Song, B.-H.; Sun, L.-H., and Zeng, A.-P. Theoretical analysis of effects of metabolic overflow and time delay on the performance and dynamic behavior of a two-stage fermentation process.* ***Biochemical Engineering Journal****. 2002;* ***11****:101-109.*
- *Yuan, Y.-J.; Li, C.; Hu, Z.-D.; Wu, J.-C., and Zeng, A.-P. Fungal elicitor-induced cell apoptosis in suspension cultures of* Taxus chinensis *var.* mairei *for taxol production.* ***Process Biochemistry****. 2002;* ***38****:193-198.*
- *Yuan, Y. J.; Ma, Z. Y.; Wu, J. C., and Zeng, A. P. Taxol-induced apoptotic cell death in suspension cultures of* Taxus cuspidata*.* ***Biotechnology Letters****. 2002;* ***24(8)****:615-618.*
- *Zander, N.; Dittrich, F.; Michaelis, K.; Tegge, W., and Frank, R. A new high-performance polypropylene-based membrane support for the parallel synthesis of peptides and small organic compounds. Peptides 2002, Proceedings 27th European Peptide Symposium. 2002; pp. 340-341.*
- *Zeng, A. P. and Biebl, H. Bulk chemicals from biotechnology: the case of 1,3-propanediol production and the new trends.* ***Advances in Biochemical Engineering – Biotechnology****. 2002;* ***74****:239-59.*
- *Zeng, A.-P.; Modak, J., and Deckwer, W.-D. Nonlinear dynamics of eucaryotic pyruvate dehydrogenase multi-enzyme complex: Decaroxylation rate, oscillations and multiplicity.* ***Biotechnology Progress****. 2002;* ***18****:1265-1276.*

Sustainable Use of Landscapes

- *Abraham, W.-R. Microbial degradation of polychlorinated biphenyls (PCBs) in the environment. In: (Ved Pal Singh and Raymond D. Stapleton Jr. editors) „Progress in Industrial Biotechnology". Elsevier, Netherlands; 2002; 36: „Biotransformations: Bioremediation Technology for Health an Environmental Protection" pp. 29-67.*

- *Abraham, W. R.; Nogales, B.; Golyshin, P. N.; Pieper, D. H., and Timmis, K. N. Polychlorinated biphenyl-degrading microbial communities in soils and sediments.* ***Current Opinion in Microbiology****. 2002;* ***5(3)****:246-253.*

- *Abraham, W. R. and Spassov, G. Biotransformations of alkaloids: a challenge.* ***Heterocycles****. 2002;* ***56(1-2)****:711-741.*

- *Abraham, W.-R.; Strömpl, C.; Bennasar, A.; Christ, R.; Vancanneyt, M.; Smit, J., and Moore, E. R. B. Phylogeny of Maricaulis Abraham et al. 1999 and proposal of* Maricaulis indicus *sp. nov.,* M. virginensis *sp. nov.,* M. parjimensis *sp. nov.,* M. washingtonensis *sp. nov., and* M. salignoratus *sp. nov.* ***International Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52****:2191-2201.*

- *Asolkar, R. N.; Kamat, V. P.; Wagner-Döbler, I., and Laatsch, H. Limnazine, the first bacterial azine derivative from* Bacillus *sp.* ***Journal of Natural Products****. 2002;* ***65****:1664-1666.*

- *Brettar, I.; Christen, R., and Höfle, M. G. Polyphasic taxonomic approach to the description of* Rheinheimera baltica *gen. nov. sp. nov., a blue colored bacterium isolated from the central Baltic sea.* ***International Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52****:1851-1857.*

- *Brettar, I.; Christen, R., and Höfle, M. G. Polyphasic taxonomic approach to the description of* Shewanella denitrificans*,sp. nov., a vigorously denitrifying bacterium isolated from the oxic-anoxic interface of the Gotland Deep in the central Baltic Sea.* ***International Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52****:2211-2217.*

- *Brettar, I. and Höfle, M. G. Close correlation between the nitrate elimination rate by denitriefication and the organic matter content in hardwood forest soils of the upper Rhine floodplain (France).* ***Wetlands****. 2002;* ***22(2)****:214-224.*

  Brettar, I.; Sanchez-Perez, J. M., and Trémoliãres, M. Nitrate elimination by denitrification in hardwood forest soils of the Upper Rhine floodplain - correlation with redox potential. ***Hydrobiologia****. 2002;* ***469****:11-21.*

- *Busse, H.-J. ; Kämpfer, P.; Denner, E. B. M.; Moore, E. R. B., and Salkinojy-Salonen, M. S.* Thermomonas haemolytica *gen. nov., sp. nov.,: a Proteobacterium from kaolin slurry.* ***International Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52****:473-483.*

- *Collins, J.; Giorgio, T.; King, P.; Alley, J.; Lauten, H.; Winter, P.; Appenzeller, A.; Scriven, J.; Jonas, R.; Berger, C.; Eichelmann, P.; Jacobsen, H.-J., and Huchzermeyer, B. A German-US faculty/intern exchange programme in biotechnology. Proceedings of the 2002 American Society for Engineering Education Annual Conference and Exposition; 2002; pp. 2227-2236.*

- *Collins, M. D.; Lawson, P. A.; Labrenz, M.; Tindall, B. J.; Weiss, N., and Hirsch, P.* Nesterenkonia lacusekhoensis *sp. nov., isolated from hypersaline Ekho Lake, East Antarctica, and emended description of the genus Nesterenkonia.* ***International Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52****:1145-1150.*

- *Dominik, K. and Höfle, M. G. Changes in bacterioplankton community structure and activity with depth in a eutrophic lake as revealed by 5S rRNA analysis.* ***Applied of Environmental Microbiology****. 2002;* ***68(7)****:3606-13.*

- *Druschel, G. K.; Labrenz, M.; Thomsen-Ebert, T.; Fowle, D. A., and Banfield, J. F. Geochemical modeling of ZnS in biofilms:An example of ore depositional processes.* ***Economic Geology and the Bulletin of the Society of Economic Geologists****. 2002;* ***97(6)****:1319-1329.*

- *Felske, A. Streamlined representational difference analysis for comprehensive studies of numerous genomes.* ***Journal of Microbiological Methods****. 2002;* ***50****:305-311.*

- *Felske, A.; Vandieken, V.; Pauling, B. V.; von Canstein, H. F., and Wagner-Döbler, I. Molecular qantification of genes encoding for green fluorescent proteins.* ***Journal of Microbiological Methods****. 2002. online: October.*

- *Golyshin, P. N.; Chernikova, T. N.; Abraham, W. R.; Lünsdorf, H.; Timmis, K. N., and Yakimov, M. M. Oleiphilaceae fam. nov., to include* Oleiphilus messinensis *gen. nov., sp nov., a novel marine bacterium that obligately utilizes hydrocarbons.* ***International Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52****:901-911.*

- *Kämpfer, P.; Witzenberger, R.; Denner, E. B. M.; Busse, H.-J., and Neef, A.* Sphingopyxis witflariensis *sp. nov., isolated from activated sludge.* ***International Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52****:2029-2034.*

- *Katsilvela, E.; Moore, E. R. B., and Kalogerakis, N. Characterization of active microbial communities degrading petroleum waste sludge. Proceedings of the International Conference „Protection and Restoration of the Environment VI", July 1-5, 2002, Skiathos. Thessaloniki, Greece: Grafima Publ.; 2002; pp. 489-496. ISBN: 960-86574-2-3.*

- *Kämpfer, P.; Jureit, C.; Albrecht, A., and Neef, A. Immission of microorgnisms from composting facilities. In: (Insam, H. et al., editors). Microbiology of Composting. Heidelberg: Springer; 2002; pp. 571-584. ISBN : 3-540-6768-X.*

- *Kämpfer, P.; Neef, A.; Salkinoja-Salonen, M. S., and Busse, H.-J.* Chelatobacter heintzii *(Auling et al. 1993) is a later subjective synonym of* Aminobacter aminovorans *(Urakami et al. 1992).* ***International Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52****:835-839.*

- *Kämpfer, P.; Witzenberger, R.; Denner, E. B. M.; Busse, H.-J., and Neef, A.* Novoshingobium hassiacum*, sp.nov., a new species isolated from an aerated sewage pond.* ***Systematic and Applied Microbiology****. 2002;* ***25****:37-45.*

- *Ledger, T.; Pieper, D. H.; Pérez-Pantoja, D., and González, B. Novel insights into interplay between xyl genes-encoded peripheral reactions and tfd genes-encoded chlorocatechol pathway for degradation of chlorobenzoates by* Ralstonia eutropha *JMP 134.* ***Microbiology UK****. 2002;* ***148****:3431-3440.*

- *Lünsdorf, H.; Wenderoth, D. F., and Abraham, W.-R. Microbial consortia in composite biofilms from acidic mining lakes.* ***Water, Air and Soil Pollution (Focus)****. 2002;* ***2****:69-79.*

- *Manaia, C. and Moore, E. R. B.* Pseudomonas thermotolerans *sp. nov., a thermotolerant species of pseudomonas.* ***International Journal of Systematic and Evolutionary Microbiology****. 2002;* ***52****:2203-2209.*

- *Maskey, R. P.; Asolkar, R. N.; Kapaun, E.; Wagner-Döbler, I., and Laatsch, H. Phytotoxic arylethylamides from limnic bacteria using a screening with microalgae.* ***Journal of Antibiotics****. 2002;* ***55****:643-649.*

- *Maskey, R. P.; Kock, I.; Shaaban, M.; Grün-Wollny, I.; Helmke, E.; Mayer, F.; Wagner-Döbler, I., and Laatsch, H. Low molecular weight oligo-hydroxybutyric acids and a monomeric amide thereof - new products from microorganisms.* ***Polymer Bulletin****. 2002;* ***49****:87-93.*

- *Muriithi, M. W.; Abraham, W. R.; Addae-Kyereme, J.; Scowen, I.; Croft, S. L.; Gitu, P. M.; Kendrick, H.; Njagi, E. N., and Wright, C. W. Isolation and in vitro antiplasmodial activities of alkaloids from* Teclea trichocarpa*: In vivo antimalarial activity and X-ray crystal structure of normelicopicine.* ***Journal of Natural Products****. 2002;* ***65(7)****:956-959.*

- *Neef, A. and Kampfer, P. Molecular identification of airborne microorganisms from composting facilities. In: (Insam, H. et al, editors). Microbioloy of Composting. Heidelberg: Springer Verlag; 2002; pp. 585-594. ISBN: 3-540-6768-X.*

- *Nelson, K. E.; Weinel, C.; Paulsen, I. T.; Dodson, R. J.; Hilbert; Martins dos Santos, V.; Fouts, D. Gill S. R.; Pop, M.; Holmes, M.; Khouri, H.; Hance, I.; Chris Lee, P.; Holtzapple, E.; Scanlan, D.; Tran, K.; Deboy, R.; Moazzez, A.; Brinkac, L.; Beanan, M.; Daugherty, S.; Kolonay, J.; Madupu, R.; Nelson, W.; White, O.; Utterback, T.; Rizzo, M.; Lee, K.; Kosack, D.; Moestl, D.; Wedler, H.; Lauber, J.; Hoheisel, J.; Strätz, M.; Heim, S.; Kiewitz, C.; Eisen, J.; Timmis, K. N.; Duesterhoft, A.; Tümmler, B., and Fraser, C. M. Complete genome sequence and comparative analysis of the metabolically versatile* Pseudomonas putida *KT2440.* ***Environmental Microbiology****. 2002;* ***4****:799-808.*
- *Pieper, D. H.; Pollmann, K.; Nikodem, P.; Gonzalez, B. , and Wray, V. Monitoring key reactions in degradation of chloroaromatics by in situ H-1 nuclear magnetic resonance: Solution structures of metabolites formed from cis-Dienelactone.* ***Journal of Bacteriology****. 2002;* ***184(5)****:1466-1470.*
- *Plumeier, I. ; Perez-Pantoja, D.; Heim, S.; Gonzalez, B., and Pieper, D. H. Importance of different tfd genes for degradation of chloroaromatics by* Ralstonia eutropha *JMP134.* ***Journal of Bacteriology****. 2002;* ***184(15)****:4054-4064.*
- *Pollmann, K.; Kaschabek, S.; Wray, V.; Reineke, W., and Pieper, D. H. Metabolism of dichloromethylcatechols as central intermediates in the degradation of dichlorotoluenes by* Ralstonia *ap. strain PS12.* ***Journal of Bacteriology****. 2002;* ***184****:5261-5274.*
- *Pöhler, I.; Wenderoth, D. F.; Wendt-Potthoff, K., and Höfle, M. G. Bakterioplankton community structure and dynamics in enclosures during bioremediation experiments in an acid miming lake.* ***Water, Air and Soil Pollution: Focus****. 2002;* ***2****:111-121.*
- *Pöhler, I.; Wenderoth, D. F.; Wendt-Potthoff, K., and Höfle, M. G. Biozönotische Struktur und Tiefenverteilung des Bakterioplanktons in einem sauren Bergbaurestsee. Tagungsband DGL Tagung 2001 Kiel. Tutzing: DGL Verlag; 2002; 1 pp. 388-391. ISBN: 3-9805678-5-0.*
- *Regenhardt, D.; Heuer, H.; Heim, S.; Fernandez, D. U.; Strömpl, C.; Moore, E. R. B., and Timmis, K. N. Pedigree and taxonomic credentials of* Pseudomonas putida *strain KT2440.* ***Environmental Microbiology****. 2002;* ***4****:912-915.*
- *Shaaban, M.; Maskey, R. P.; Wagner-Döbler, I., and Laatsch, H. Pharacine, a natural p-cyclophane and other new indol derivatives from* Cytophaga sp. *strain AM13.1.* ***Journal of Natural Products****. 2002;* ***65****:1660-1663.*
- *Skiba, A.; Hecht, V., and Pieper, D. H. Formation of protoanemonin from 2-chlorocis, cis-muconate by the combined action of muconate cycloisomerase and muconolactone isomerase.* ***Journal of Bacteriology****. 2002;* ***184****:5402-5409.*
- *Timmis K.N.* Pseudomonas putida*: a cosmopolitan opportunist par excellence.* ***Environmental Microbiology****. 2002;* ***4****:779-781.*
- *Trefault, N.; Clement, P.; Manzano, M.; Pieper, D. H., and Gonzalez, B. The copy number of the catabolic plasmid pJP4 affects growth of* Ralstonia eutropha *JMP134 (pJP4) on 3-chlorobenzoate.* ***FEMS Microbiology Letters****. 2002;* ***212(1)****:95-100.*
- *Vasquez, M.; Gruttner, C.; Möeller, B., and Moore, E. R. B. Limited selection of sodium channel blocking toxin producing bacteria from paralytic shellfish toxin-contaminated mussels (*Aulacomya ater*).* ***Research in Microbiology****. 2002;* ***153****:333-338.*
- *Von Canstein, H.; Kelly, S.; Li, Y., and Wagner-Döbler, I. Species diversity i mproves the efficiency of mercury-reducing biofilms under changing environmental conditions.* ***Applied and Environmental Microbiology****. 2002;* ***68(6)****:2829-2837.*
- *Von Canstein, H.; Li, Y.; Leonhauser, J.; Haase, E.; Felske, A.; Deckwer, W. D., and Wagner-Döbler, I. Spatially oscillating activity and microbial succession of mercury-reducing biofilms in a technical-scale bioremediation system.* ***Applied and Environmental Microbiology****. 2002;* ***68(4)****:1938-1946.*
- *Wagner-Döbler, I. Mercury remediation using natural and recombinant microbes.* ***Marine Biotechnology****; 2002;* ***8****:189-201.*
- *Wagner-Döbler, I.; Beil, W.; Lang, S.; Meiners, M., and Laatsch, H. Integrated approach to explore the potential of marine microorganisms for the production of bioactive metabolites.* ***Advances in Biochemical Engeering/Biotechnology****; 2002;* ***74****:207-38.*
- *Wagner-Döbler, I.; Rheims, H.; Felske, A.; Pukall, R., and Tindall, B.* Jannaschia helgolandensis*, gen. nov., sp. nov., a novel abundant member of the marine* Roseobacter *clade from the North Sea.* ***International Journal of Systematic and Evolutionary Microbiology****. 2002; DOI 10.1099/ijs.0.02377-0.*
- *Wagner-Döbler, I.; von Canstein, H.; Leonhäuser, J.; Li, Y., and Deckwer, W-D. Prozessintegrierte Quecksilberentfernung aus Abwässern der Chloralkali-Elektrolyse durch Mikroorganismen.* ***Chemie Ingenieur Technik****. 2002;* ***74****:1-8.*
- *Wagner-Döbler, I.; Von Canstein, H.; Li, Y.; Leonhäuser, J., and Deckwer, W. D. Process-integrated removal of mercury from chloralkali electrolysis by microorganisms.* ***Chemie Ingenieur Technik****. 2002;* ***74(4)****:504-508.*
- *Weinbauer, M. G.; Fritz, I.; Wenderoth, D. F., and Höfle, M. G. Simultaneous extraction from bacterioplankton of total RNA and DNA suitable for quantitative structure and function analyses.* ***Applied and Environmental Microbiology****. 2002;* ***68(3)****:1082-7.*
- *Weinbauer, M. G. and Höfle, M. G, authors. Quantification of nucleic acids from aquatic environments using green-fluorescent dyes and microtiter plates. Manual of Molecular Microbial Ecology. Dordrecht, The Netherlands: Kluwer Academic Publishers; 2002; 2.1.3 pp. 1-10.*
- *Weinbauer, M. G.; Winter, C., and Höfle, M. G. Reconsidering transmission electron microscopy based estimates of viral infection of bacterio-plankton using conversion factors derived from natural communities.* ***Aquatic Microbial Ecology****. 2002;* ***27(2)****:103-110.*
- *Wenderoth, D. F.; Rosenbrock, P.; Pieper, D., and Höfle, M. G. Assessment of population dynamics of specific in groundwater bioaugmentation experiments by two different molecular techniques.* ***Water, Air and Soil Pollution: Focus****. 2002;* ***2****:195-203.*
- *Witzenberger, R.; Neef, A., and Kämpfer, P. Abundance and ecophysiology of sphingomonads in municipalactivated sludges.* ***Water Intelligence Online****. 2002; 1(12). (http://www.iwaponline.com/wio/2002/12/wio200212014.htm)*
- *Yakimov, M. M.; Giuliano, L.; Crisafi, E.; Chernikova, T. N.; Timmis, K. N., and Golyshin, P. N. Microbial community of a saline mud volcano at San Biagio- Belpasso, Mt. Etna (Italy).* ***Environmental Microbiology****. 2002;* ***4(5)****:249-256.*
- *Zarnowski, R.; Felske, A.; Ellis, R. J.; Genus, J. M. C.; Zarnowska, E. D.; Lewicka, T., and Pietr., S. J. A Methylobacterium-like symbiont from algal crusts covering African electric tractions.* ***Journal of Applied Microbiology****. 2002;* ***93****:1012-1019.*
- *Zielinski, M.; Backhaus, S., and Hofer, B. The principal determinants for the structure of the substrate-binding pocket are located within a central core of a biphenyl dioxygenase subunit.* ***Microbiology****. 2002;* ***148****:2439-2448.*

Technological Platforms

- *Edrada, R. A. ; Ebel, R.; Supriyono, A.; Wray, V.; Schupp, P.; Steube, K.; van Soest, R., and Proksch, P. Swinhoeiamide A, a new highly active Calyculin derivative from the marine sponge,* Theonella swinhoei. ***Journal of Natural Products***. *2002;* ***65****:1168-1172.*

- *Edrada, R. A.; Heubes, M.; Brauers, G.; Wray, V.; Berg, A.; Gräfe, U.; Wohlfahrt, M.; Mühlbacher, J.; Schaumann, K.; Sudarsono; Bringmann, G., and Proksch, P. Online analysis of xestodecalactones A-C, novel bioactive metabolites from the fungus* Penicillium cf. montanese *and their subsequent isolation from the sponge* Xestospongia exigua. ***Journal of Natural Products***. *2002;* ***65****:1598-1604.*

- *Fester, T.; Hause, B.; Schmidt, D.; Halfmann, K; Schmidt, J.; Wray, V.; Hause, G., and Strack, D. Occurence and localization of apocarotenoids in arbuscular mycorrhizal plant roots.* ***Plant Cell Physiology***. *2002;* ***43(3)****:256-265.*

- *Jadulco, R.; Brauers, G.; Edrada, R. A.; Ebel, R.; Wray, V.; Sudarsono, and Proksch, P. New metabolites from sponge-derived fungi* Curvularia lunata *and* Cladosporium herbarum. ***Journal of Natural Products***. *2002;* ***65(5)****:730-733.*

- *Kim, W. S.; Schollmeyer, M.; Nimtz, M.; Wray, V., and Geider, K. Genetics of biosynthesis and structure of the capsular exopolysaccharide from the Asian pear pathogen* Erwinia pyrifoliae. ***Microbiology***. *2002;* ***148****:4015-4024.*

- *Landtag, J.; Baumert, A.; Degenkolb, T.; Schmidt, J.; Wray, V.; Scheel, D.; Strack, D., and Rosahl, S. Accumulation of tyrosol glucoside in transgenic potato plants expressing a parsley tyrosine decarboxylase.* ***Phytochemistry***. *2002;* ***60****:683-689.*

- *Philp, J. C.; Kuyukina, M. S.; Ivshina, I. B.; Dunbar, S.; Ritchkova, M. I.; Lang, S., and Wray, V. Alkanotrophic* Rhodococcus ruber *as a biosurfactant producer.* ***Applied Microbiology and Biotechnology***. *2002;* ***59****:318-324.*

- *Pieper, D. H. ; Pollmann, K.; Nikodem, P.; Gonzalez, B., and Wray, V. Monitoring key reactions in degradation of chloroaromatics by in situ H-1 nuclear magnetic resonance: Solution structures of metabolites formed from cis-Dienelactone.* ***Journal of Bacteriology***. *2002;* ***184(5)****:1466-1470.*

- *Riaz, M.; Krohn, K.; Wray, V., and Malik, A. Dicoumarinyl ether Ggycoside from the roots of* Daphne oleoides. ***European Journal of Organic Chemistry***. *2002;* ***(8)****:1436-1438.*

- *Schupp, P.; Proksch, P., and Wray, V. Further new staurosporine derivatives from the ascidian* Eudistoma toealensis *and its predatory flatworm* Pseudoceros *sp.* ***Journal of Natural Products***. *2002;* ***65(3)****:295-298.*

- *Wang, B. G.; Ebel, R.; Wang, C. Y.; Wray, V., and Proksch, P. New methoxylated aryltetrahydronaphthalene lignans and a norlignan from* Aglaia cordata. ***Tetrahedron Letters***. *2002;* ***43****:5783-5787.*

Biotech Facilities

- *Collins, J.; Giorgio, T.; King, P.; Alley, J.; Lauten, H.; Winter, P.; Appenzeller, A.; Scriven, J.; Jonas, R.; Berger, C.; Eichelmann, P.; Jacobsen, H.-J., and Huchzermeyer, B. A German-US faculty/intern exchange programme in biotechnology. Proceedings of the 2002 American Society for Engineering Education Annual Conference and Exposition; 2002; pp. 2227-2236.*

- *Elias, C. B.; Carpentier, E.; Durocher, Y.; Bisson, L.; Wagner, R., and Kamen, A. Improving glucose and glutamine metabolism of human HEK 293 and* Trichoplusia ni *insect cells engineered to express a cytosolic pyruvate carboxylase enzyme.* ***Biotechnology Progress***. *2002; ID-10.1021/bp025572x.*

- *Gouda, M. K.; Kleeberg, I.; van den Heuvel, J.; Müller, R.-J., and Deckwer, W.-D. Production of a polyester degrading extracellular hydrolase from* Thermomonspora fusca. ***Biotechnology Progress***. *2002;* ***18****:927-929.*

- *Hoffmann, F.; Weber, J., and Rinas, U. Metabolic adaptation of* Escherichia coli *during temperature-induces recombinant protein synthesis. 1. Readjustment of metabolic enzyme synthesis.* ***Biotechnology and Bioengineering***. *2002;* ***80****:313-319.*

- *Irani, N.; Beccaria, A. J., and Wagner, R. Expression of recombinant cytoplasmic yeast pyruvate carboxylase for the improvement of the production of human erythropoietin by recombinant Bhk-21 cells.* ***Journal of Biotechnology***. *2002;* ***93(3)****:269-282.*

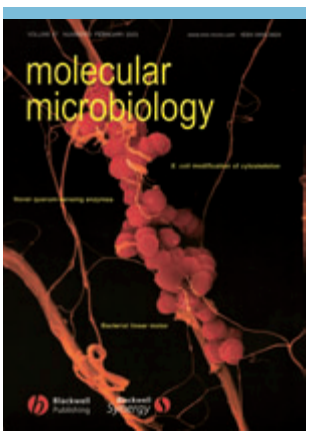
- *Silveira, M. M. and Jonas, R. The biotechnological production of sorbitol.* ***Applied Microbiology and Biotechnology***. *2002;* ***59****:400-408.*

- *Vallejo, L. F.; Brokelmann, M.; Marten, S.; Trappe, S.; Cabrera-Crespo, J.; Hoffmann, A.; Gross, G.; Weich, H. A., and Rinas, U. Renaturation and purification of bone morphogenetic protein-2 produced as inclusion bodies in high-cell-density cultures of recombinant* Escherichia coli. ***Journal of Biotechnology***. *2002;* ***94(2)****:185-194.*

- *Vallejo, L. F. and Rinas, U. Strategies for refolding inclusion body proteins. (Villaverde, A., editor). Recent research development in biotechnology and bioengineering/protein production in bacterial cell factories. Trivandrum -695 023, Kerala, India: Research Signpost; 2002; pp. 59-71.*

- *Weber, J.; Hoffmann, F., and Rinas, U. Metabolic adaptation of* Escherichia coli *during temperature-induces recombinant protein synthesis. 2. Redirection of metabolic fluxes.* ***Biotechnology and Bioengineering***. *2002;* ***80****:320-330.*

- *Welzel, K.; Müller, R.-J., and Deckwer, W.-D. Enzymatischer Abbau von Polyester-Nanopartikeln.* ***Chemie Ingenieur Technik***. *2002;* ***74(10)****:1496-1500.*



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Publications 2003

Infection and Immunity

- *Beer, C.; Buhr, P.; Hahn, H.; Laubner, D., and Wirth, M. Gene expression analysis of murine cells producing amphotropic mouse leukemia virus at a cultivation temperature of 32 and 37°C.* ***Journal of General Virology****. 2003;* ***84****:1677-1686.*

- *Beer, C.; Meyer, A.; Müller, K., and Wirth, M. The temperature stability of mouse retroviruses depends on the cholesterol levels of viral lipid shell and cellular plasma membrane.* ***Virology****. 2003;* ***308****:137-146.*

- *Bergmann, S.; Wild, D.; Diekmann, O.; Frank, R.; Bracht, D., and Hammerschmidt, S. Binding of human plasmin(ogen) to surface displayed -enolase is mediated via two binding sites in Eno of* Streptococcus pneumoniae. ***Molecular Microbiology****. 2003;* ***49(2)****:411-423.*

- *Bode, H. B. and MüllerR. Possibility of bacterial recruitment of plant genes associated with the biosynthesis of secondary metabolites.* ***Plant Physiology****. 2003;* ***132****:1153-1161.*

- *Bode, H. B.; Irschik, H.; Wenzel, S. C.; Reichenbach, H.; Müller, R., and Höfle, G. The leupyrrins: a structurally unique family of secondary metabolites from the Myxobacterium* Sorangium cellulosum. ***Journal of Natural Products****. 2003;* ***66****:1203-1206.*

- *Bode, H. B.; Zeggel, B.; Silakowski, B.; Wenzel, S. C.; Reichenbach, H., and Müller, R. Steroid biosynthesis in procaryotes: identification of myxobacterial steroids and cloning of the first bacterial 2,3(s)-oxidosqualen cyclase from the myxobycterium* Stigmatella aurantiaca. ***Molecular Microbiology****. 2003;* ***47****:471-481.*

- *Bode, J.; Götze, S.; Ernst, E.; Hüsemann, Y.; Baer, A.; Seibler, J., and Mielke, C. Architecture and utilization of highly-expressed genomic sites. In: (Makrides, S., editor). New Comprehensive Biochemistry - Gene Transfer and Expression in Mammalian Cells: Elsevier; 2003; Chap.20,pp.551-572.*

- *Borsutzky, S.; Fiorelli, V.; Ebensen, T.; Tripiciano, A.; Rharbaoui, F.; Scoglio, A.; Link, C.; Nappi, F.; Morr, M.; Buttó, S.; Cafaro, A.; Mühlradt, P. F.; Ensoli, B., and Guzmán, C. A. Efficient mucosal delivery of the HIV-1 Tat protein using the synthetic lipopeptide MALP-2 as adjuvant.* ***European Journal of Immunology****. 2003;* ***33****:1548-1556.*

- *Breitbach, K.; Rottner, K.; Klocke, S.; Rohde, M.; Jenzora, A.; Wehland, J., and Steinmetz, I. Actin-based motility of* Burkholderia pseudomallei *involves the Arp2/3 complex, but not N-WASP and Ena/VASP proteins.* ***Cellular Microbiology****. 2003;* ***5(6)****:385-393.*

- *Buer, J. and Balling, R. Mice, microbes and models of infection.* ***Nature Reviews Genetics****. 2003;* ***4****:195-205.*

- *Deckert, M.; Lutjen, S.; Leuker, C. E.; Kwok, L.-Y.; Strack, A.; Müller, W.; Wagner, N., and Schlüter, D. Mice with neonatally induced inactivation of the vascular cell adhesion molecule-1 fail to control the parasite in* Toxoplasma encephalitis. ***European Journal of Immunology****. 2003;* ***33(5)****:1418-1428.*

- *Dietrich, G.; Spreng, S.; Favre, D.; Viret, J.-F., and Guzmán, C. A. Live attenuated bacteria as vectors to deliver plasmid DNA vaccines.* ***Current Opinion in Molecular Therapeutics****. 2003;* ***5****:10-19.*

- *Dietz-Pfeilstetter, A.; Arndt, N.; Kay, V., and Bode, J. Molecular structure and regulatory potential of a T-DNA integration site in petunia.* ***Transgenic Research****. 2003;* ***12****:83-99.*

- *Dinkla, K.; Rohde, M.; Jansen, W. T. M.; Carapetis, J. R.; Chhatwal, G. S., and Talay, S. R. Streptococcus pyogenes recrutis collagen via surface bound fibronectin: a novel colonisation and immune evasion mechanism.* ***Molecular Microbiology****. 2003;* ***47****:861-869.*

- *Dinkla, K.; Rohde, M.; Jansen, W. T. M.; Kaplan, E. L.; Chhatwal, G. S., and Talay, S. R. Rheumatic fever associated* Streptococcus pyogenes *isolates aggregate collagen.* ***Journal of Clinical Investigation****. 2003;* ***111(12)****:1905-1912.*

- *Duvar, S.; Berlin, J.; Ziehr, H., and Conradt, H. S. Modulation of the glycosylation repertoire of recombiant human EPO expressing model cell lines under different culture conditions. Proceedings of the 18th ESACT-Meeting-Granada. 2003.*

- *Erck, C.; McLeod, R., and Wehland, J. Cloning and genomic organisation of the TTL gene on mouse chromosome 2 and human chromosome 2q13.* ***Cytogenetic Genome Research****. 2003;****101****:47-53*

- *Fingerle-Rowson, G.; Petrenko, O.; Metz, C. N.; Forsthuber, T. G.; Mitchell, R.; Huss, R.; Moll, U.; Müller, W., and Bucala, R. The p53-dependent effects of macrophage migration inhibitory factor revealed by gene targeting.* ***Proceedings of the National Academy of Sciences****, USA. 2003;* ***100****:9354-9359.*

- *Franke, D.; Lorbach, V.; Esser, S.; Dose, C.; Sprenger, G. A.; Halfar, M.; Thömmes, J.; MüllerR.; Takors, R., and Müller, M. (S,S)-2,3-Dihydroxy-2,3-dihydrobenzoic acid: Microbial access with engineered cells of* Escherichia coli *and applicability as starting material in natural-product synthesis.* ***Chemical European Journal****. 2003;* ***9****:4188-4196.*

- *Franzke, A.; Piao, W.; Lauber, J.; Gatzlaff, P.; Könecke, C.; Hansen W.; Schmitt-Thomsen, A.; Herstenstein, B.; Buer, J., and Ganser, A. G-CSF as immune regulator in t-cells expressing the G-CSF receptor: implications for transplantation and autoimmune diseases.* ***Blood****. 2003;* ***102****:734-739.*

- *Goldmann, O. ; Chhatwal, G. S., and Medina, E. Immune mechanisms underlying host susceptibility to group A streptococcal infections.* ***Journal of Infectious Diseases****. 2003;* ***187****:854-861.*

- *Grenklo, S.; Geese, M.; Lidberg, U.; Wehland, J.; Karlsson, R., and Sechi, A. S. Critical role for profilin: actin in the intracellular motility of* Listeria monocytogenes. ***EMBO Reports****. 2003;* ***4****:1-7.*

- *Götze, S. ; Gluch, A.; Benham, C., and Bode, J. Computational and in vitro analysis o destabilized DNA regions in the interferon gene cluster: the potential of predicting functional gene domains.* ***Biochemistry****. 2003;* ***42****:154-166.*

- *Goetze, S.; Huesemann, Y.; Baer, A., and Bode, J. Functional characterization of transgene integration patterns by halo-fluorescence in situ hybridization: electroporation versus retroviral infection.* ***Biochemistry****. 2003;* ***42 (23)****:7035-7043*

- *Heilmann C., Thumm G., Chhatwal G. S., Hartleib J., Uekotter A., Peters G. Identification and characterization of a novel autolysin (Aae) with adhesive properties from* Staphylococcus epidermidis. ***Microbiology****. 2003;* ***149****:2769-2778*

- *Höfle, G.; Glaser, N.; Karama, U.; Leibold, T.; Sasse, F., and Steinmetz, H. Semisynthesis and degradation of the tubulin inhibitors epothilone and tubulysin.* ***Pure and Applied Chemistry****. 2003;* ***75****:167-178.*

- *Hollister, J.; Conradt, H. S., and Jarvis, D. L. Evidence for a sialic acid salvaging pathway in lepidopterian insect cells.* ***Glycobiology****. 2003;* ***13(6)****:487-495.*

- *Jansen, R.; Kunze, B.; Reichenbach, H., and Höfle, G. +Chondrochloren A and B, new ß-amino styrenes from* Chondromyces crocatus *(Myxobacteria).* ***European Journal of Organic Chemistry****. 2003; 2684-2689.*

- *Karama, U. and Höfle, G. Synthesis of epothilone 16,17-alkyne analogs by replacement of the C13-C15(O)-ring segment of natural epothilone C.* ***European Journal of Organic Chemistry****. 2003;* ***(6)****:1042-1049.*

- *Kaverina, I.; Stradal, T. E B., and Gimona, M. Podosome formation in cultured A7r5 vascular smooth muscle cells requires Arp2/3-dependent de-novo actin polymerization at discrete microdomains.* ***Journal of Cell Science.*** *2003;* ***116****:4915-4924.*

- *Köster, M.; Lykke-Andersen, S.; Elnakady, Y. A.; Gerth, K.; Washausen, P.; Höfle, G.; Sasse, F.; Kjems, J., and Hauser, H. Ratjadones inhibit nuclear export by blocking DRM1/exportin 1.* ***Experimental Cell Research****. 2003;* ***286****:321-331.*

- *Kresse, A. U.; Dinesh, S. D.; Larbig, K., and Römling, U. Impact of large chromosomal inversions on the adaption and evolution of* Pseudomonas aeruginosa *chronically colonizing cystic fibrosis lungs.* ***Molecular Microbiology****. 2003;* ***47(1)****:145-158.*

- *Kretschmer, K.; Joungebloud, A.; Stopkowicz, J.; Störmann, B.; Hoffmann, R., and Weiss, S. Antibody repertoire and geneexpression profile: implifications on different developmental and functional traits of splenic and peritioneal B-1 lymphocytes.* ***Journal of Immunology****. 2003;* ***171(3)****:1192-1201.*

- *Kröger, A.; Dallügge, A.; Kirchhoff, S., and Hauser, H. IRF-1 reverts the transformed phenotype of oncogenically transformed cells* in vitro *an* in vivo. ***Oncogene****. 2003;* ***22(7)****:1045-1056.*

- *Kwissa, M.; Kröger, A.; Hauser, H.; Reimann, J., and Schirmbeck, R. Defining conditions for cytokine-facilitated priming of CD8+ T cell responses by DNA vaccination.* ***Journal of Molecular Medicine****. 2003;* ***81****:91-101.*

- *Lindenmaier, W. Gentechnik und Schöpfungsglaube. In: (Babke, H.-G. Fritsche A., editors). Gerechtigkeit - ein globaler Wert. München; 2003; pp. 163-177.*

- *Lipps, H. J.; Jenke, A. C. W.; Nehlsen, K.; Scinteie, M.; Stehle, I. M., and Bode, J. Chromosome-based vectors for gene therapy.* ***Gene****. 2003;* ***304****:23-33.*

- *Machner, M. P.; Frese, S.; Schubert, W.-D.; Orian-Rousseau, V.; Gherardi, E.; Wehland, J.; Niemann, H. H., and Heinz, D. W. Aromatic amino acids at the surface of In1B are essential for host cell invasion by* Lysteria monocytogenes. ***Molecular Microbiology****. 2003;* ***48****:1525-1536.*

- *Maiorino, M.; Bosello, V.; Ursini, F.; Foresta, C.; Garolla, A.; Scapin, M.; Sztajer, H., and Flohé, L. Genetic variations of gpx-4 and male infertility in humans.* ***Biology of Reproduction****. 2003;* ***68****:1134-1141.*

- *Manitz, M. P.; Horst, B.; Seeliger, S.; Strey, A.; Skryabin, B. V.; Gunzer, M.; Frings, W.; Schonlau, F.; Roth, J.; Sorg, C., and Nacken, W. Loss of S100A9 (MRP14) results in reduced interleukin-8-induced CD11b surface expression, a polarized microfilament system, and diminished responsiveness to chemoattractants in vitro.* ***Molecular and Cellular Biology****. 2003;* ***23(3)****:1034-1043.*

- *Mascher, T; Zähner, D; Balmelle, N.; de Saizieu, A., and Hakenbeck, R. The* Streptococcus pneumoniae *cia regulon: CiaR target sites and transcription profile analysis.* ***Journal of Bacteriology****. 2003;* ***185(1)****:60-70.*

- *Mataraza, J. M.; Briggs, M. W.; Li, Z.; Frank, R., and Sacks, D. B. Identification and characterization of the Cdc42-binding site of IQGAP1.* ***Biochemical and Biophysical Research Communications****. 2003;* ***305****:315-321.*

- *Matussek, A. ; Lauber, J.; Bergau, A.; Hansen, W.; Rohde, M.; Dittmar, K. E.; Gunzer, M.; Mengel, M.; Gatzlaff, P.; Buer, J., and Gunzer, F. Molecular and functional analysis of Shiga toxin induced response patterns in human vascular endothelial cells.* ***Blood****. 2003;* ***102(4)****:1323-1332.*

- *McArthur, J. D.; West, N. P.; Cole, J. N.; Jungnitz, H.; Guzmán, C. A.; Chin, J.; Lehrbach, P. R.; Djordjevic, S. P., and Walker, M. J. An aromatic amino acid auxotrophic mutant of* Bordetella bronchiseptica *is attenuated and immunogenic in a mouse model of infection.* ***FEMS Microbiology Letters****. 2003;* ***221****:7-16.*

- *Medina, E.; Rohde, M., and Chhatwal, G. S. Intracellular survival of* Streptococcus pyogenes *in polymorphonuclear cells results in increased bacterial virulence.* ***Infection and Immunity****. 2003;* ***71****:5376-5380*

- *Medina, E.; Goldmann, O.; Toppel, A.; Rohde, M., and Chhatwal, G. S. Exploitation of host phagocytic cells by* Streptococcus pyogenes *for persistence an systemic dissemination.* ***Journal of Infectious Diseases****. 2003;* ***187****:597-603.*

- *Mothana, R. A. A.; Awadh Ali, N. A.; Jansen, R.; Wegner, U.; Mentel, R., and Lindequist, U. Antiviral lanostanoid triterpenes from the fungus* Ganoderma pfeifferi. ***Fitoterapia****. 2003;* ***74(1-2)****:177-180.*

- *Müller, P. P.; Wirth, D.; Unsinger, U., and Hauser, H. Genetic approaches to recombinant protein production in mammalian cells. In: (Vinci, V. A. and Parekh, S. R., editors). Handbook of Industrial Cell Culture: Mammalian, Microbial and Plant Cells. Humana Press; 2003; pp. 21-50.*

- *Mundt, S.; Kreitlow, S., and Jansen, R. Fatty acids with antibacterial activity from the cyanobacterium* Oscillatoria redekei *HUB 051.* ***Journal of Applied Phycology****. 2003;* ***15(2)****:263-267.*

- *Müthing, J.; Kemminer, S. E.; Conradt, H. S.; Sagi, D.; Nimtz, M.; Karst, U., and Peter-Katalinic, J. Effects of buffering conditions and culture pH on production rates and glycosylation of clinical phase I anti-melanoma mouse IgG3 monoclonal antibody R24.* ***Biotechnology and Bioengineering****. 2003;* ***83(5)****:321-334.*

- *Nakagawa, H.; Miki, H.; Nozumi, M.; Takenawa, T.; Miyamoto, S.; Wehland, J., and Small, J. V. IRSp53 is co-localised with WAVE2 at the tips of protruding lamellipodia and filopodia independently of Mena.* ***Journal of Cell Science****. 2003;* ***116****:2577-2583.*

- *Noguera-Obenza, M.; Ochoa, T. J.; Gomez, H. F.; Guerrero, M. L.; Herrera-Insua, I.; Morrow, A. L.; Ruiz-Palacios, G.; Pickering, L. K.; Guzmán, C. A., and Cleary, T. G. Human milk secretory antibodies against attching and effacing* Escherchia coli *antigens.* ***Emerging Infectious Diseases*** *(United States). 2003;* ***9****:545-551.*

- *Pulz, M.; Matussek, A.; Monazahian, M.; Tittel, A.; Nikolic, E.; Hartmann, M.; Bellin, T.; Buer, J., and Gunzer, F. Comparison of a shiga toxin enzyme-linked immunosorbent assay and two different types of PCR for detection of shiga toxin-producing* Escherichia coli *in human stool specimens.* ***Journal of Clinical Microbiology****. 2003;* ***41(10)****:4671-4675.*

- *Ranson, T.; Vosshenrich, C. A.; Corcuff, E.; Richard, O.; Müller, W., and Di Santo, J. P. IL-15 is essential mediator of peripheral NK cell homeostasis.* ***Blood****. 2003;* ***101****:4887-4893.*

- *Reinscheid, D. J.; Ehlert, K.; Chhatwal, G. S., and Eikmanns, B. J. Functional analysis of a Pcs-deficient mutant of group B streptococcus.* ***FEMS Microbiology Letters****. 2003;* ***221****:73-79*

- *Rodriguez, A.; Troye-Blomberg, M.; Lindroth, K.; Ivanyi, J.; Singh, M., and Fernandez, C. B- and C-cell responses to the mycobacterium surface antigen PstS-1 in the respiratory tract and adjacent tissues. Role of adjuvants and routes of immunization.* ***Vaccine****. 2003;* ***21****:458-467.*

- *Rohde, M.; Müller, E.; Chhatwal, G. S., and Talay, S. R. Host cell caveolae act as an entry-port for Group A streptococci.* ***Cellular Microbiology****. 2003;* ***5****:323-342.*

- *Salunkhe, P.; von Götz, F.; Wiehlmann, L.; Lauber, J.; Buer, J., and Tümmler, B. Gene-chip expression analysis of the response of* Pseudomonas aeruginosa *to paraquat induced superoxide stress.* ***Genome Letters****. 2003;* ***4****:165-174.*

- *Sasse, F.; Steinmetz, H.; Höfle, G., and Reichenbach, H. Archazolid A, new cytotoxic macrolactones from* Archangium gephyra *(Myxobacteria).* ***Journal of Antibiotics****. 2003;* ***56(6)****:520-525.*

- *Scheele, U.; Alves, J.; Frank, R.; Duwel, M.; Kalthoff, C., and Ungewickell, E. J. Molecular and functional characterization of clathrin and AP-2 binding determinants within a disordered domain of auxilin.* ***Journal of Biological Chemistry****. 2003;* ***278(28)****:25357-25368.*
- *Schomburg, L.; Schweizer, U.; Holtmann, B.; Flohé, L.; Sendtner, M., and Köhrle, J. Gene disruption discloses role of selenoprotein P in selenium delivery to target tissues.* ***Biochemical Journal****. 2003;* ***370(2)****:397-402.*
- *Schrader, A. J.; Lauber, J.; Lechner, O.; Heidenreich, A.; Hofmann, R., and Buer, J. Application of real-time RT-PCR in urologic oncology.* ***Journal of Urology****. 2003;* ***169****:1858-1864.*
- *Schubert, W.-D. and Heinz, D. W. Details der Wechselwirkung zwischen Bakterium und Mensch.* ***Laborwelt****. 2003;* ***(1)****:16.*
- *Schulze, K.; Medina, E.; Chhatwal, G. S., and Guzmán, C. A. Stimulation of long-lasting protection against* Streptococcus pyogenes *after intranasal vaccination with non adjuvanted fibronectin-binding domain of the SfbI protein.* ***Vaccine****. 2003;* ***21****:1967-1973.*
- *Spitzer, D.; Dittmar, K. E.; Rohde, M.; Hauser, H., and Wirth, D. Green fluorescent protein-tagged retroviral envelope protein for analysis of virus-cell interactions.* ***Journal of Virology****. 2003;* ***77****:6070-6075.*
- *Stock, M.; Schafer, H.; Stricker, S.; Gross, G.; Mundlos, S., and Otto, F. Expression of galectin-3 in skeletal tissues is controlled by Runx2.* ***Journal of Biological Chemistry****. 2003;* ***278****:17360-17367.*
- *Stradal, T. B.; Sechi, A. S.; Wehland, J., and Rottner, K. The cytoskeleton. In: Essential Cell Biology Vol. 1- Cell Structure: Practical Approach Series: Oxford University Press; 2003.*
- *Streetz, K.; Wüstefeld, T.; Klein, C.; Kallen, K. J.; Tronche, F.; Betz, U.; Schütz, G.; Manns, M. P.; Müller, W., and Trautwein, C. Lack of gp 130 expression in hepatocytes promotes liver injury.* ***Gastroentorology****. 2003;* ***125****:532-543.*
- *Streetz, K. L.; Tacke, F.; Leifeld, L.; Wüstefeld, T.; Graw, A.; Klein, C.; Kamino, K.; Spengler, U.; Kreipe, H.; Kubicka, S.; Müller, W.; Manns, M. P., and Trautwein, C. Interleukin 6/gp130-dependent pathways are protective during chronic liver diseases.* ***Hepatology****. 2003;* ***38****:218-229.*
- *Toppel, A.; Rasmussen, M.; Medina, E., and Chhatwal, G. S. Contribution of protein G-related ¿2-macroglobulin binding protein to bacterial virulence in a mouse skin model of group A streptococcal infection.* ***Journal of Infectious Diseases****. 2003;* ***187****:1694-1703.*
- *Voigt, J. and Frank, R. 14-3-3 proteins are constituents of the insoluble glycoprotein framework of the clamydomonas cell wall.* ***Plant Cell****. 2003;* ***15(6)****:1399-1413.*
- *Vosshenrich, C.; Cumano, A.; Müller, W.; Di Santo, J. P., and Vieira, P. Thymic stromal-derived lymphopoietin distinguishes fetal from adult B cell development.* ***Nature Immunology****. 2003;* ***4(8)****:773-779.*
- *Walter, U.; Toepfer, T.; Dittmar, K. E. D.; Kretschmer, K.; Lauber, J.; Weiß, S.; Servos, G.; Lechner, O.; Scherbaum, W. A.; Bornstein, S. R.; von Boehmer, H., and Buer, J. Pancreatic NOD beta-cells express MHC class II protein and frequency of I-A(g7) mRNA expressing beta-cells strongly increase during progression to autoimmune diabetes.* ***Diabetologia****. 2003; 4****6(8)****:1106-1114.*
- *Weiss, S. Transfer of eukayotic expression plasmids to mammalian hosts by attenuated* Salmonella sp. ***Journal of Medical Microbiology****. 2003;* ***293****:95-106.*
- *Werhahn, W.; Jänsch, L., and Braun, H. P. Identification of novel subunits of the tom complex from* Arabidopsis thaliana. ***Plant Physiology****. 2003;* ***41****:407-416.*
- *Wiethe, C.; Dittmar, K.; Doan, T.; Lindenmaier, W., and Tindle, R. Provision of 4-1bb ligand enhances effector and memory ctl responses generated by immunization with dendritic cells a human tumor-associated antigen.* ***Journal of Immunology****. 2003;* ***170****:2912-2922.*
- *Wüstefeld, T.; Klein, C.; Streetz, K. L.; Betz, U.; Lauber, J.; Buer, J.; Manns, M. P.; Müller, W., and Trautwein, C. IL6/GP130-dependent pathways are protective during liver regeneration.* ***Journal of Biological Chemistry****. 2003;* ***278****:11281-11288.*
- *Zähner, D.; Kaminski, K.; van der Linden, M.; Mascher, T.; Merai, M., and Hakenbeck, R. The ciaR/ciaH regulatory system of* Streptococcus pneumoniae *is involved in beta-lactam resistance and genetic competence. In: Regulatory Networks in Prokaryotes. Norfolk: Horizon Press; 2003; pp. 41-46.*
- *Zander, N.; Gerhardt, J., and Frank, R. Polystyrylsulfonyl-3-nitro-1H-1,2,4-triazolide-resin: a new solid-supported reagent for the esterification of amino acids.* ***Tetrahedron Letters****. 2003;* ***44(35)****:6557-6560.*
- *zur Lage, S.; Goethe, R.; Darji, A.; Valentin-Weigand, P., and Weiss, S. Activation of macrophages and interference with CD4+ T cell stimulation by* Mycobacterium avium *ssp.* paratuberculosis *and* Mycobacterium avium *ssp.* avium. ***Immunology****. 2003;* ***108(1)****:62-69.*

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- *Alter-Koltunoff, M.; Ehrlich, S.; Dror, N.; Azriel, A.; Eilers, M.; Hauser, H.; Bowen, H.; Barton, C.-H.; Tamura, T.; Ozato, K., and Levi, B.-Z. Nramp 1 mediated innate resistance to intraphagosomal pathogens is regulated by IRF-8, PU.1 and Miz-1.* ***Journal of Biological Chemistry****. 2003.*
- *Barthold, M.; Majore, I.; Fargali, S.; Stahl, F.; Schulz, R.; Lose, S.; Mayer, H., and Jäger, V. 3D-cultivation and characterisation of osteogenic cells for the production of highly viable bone tissue implants. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers. 2003.*
- *Bassani Molinas, M. M.; Nelving, A.; Beer, C.; Hesse, F.; Wirth, M.; Durocher, Y.; Kamen, A., and Wagner, R. Intracellular nucleotide pools for optimizing product-oriented transient tranfection of HEK293 cells in suspension. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers. 2003.*
- *Bode, J.; Götze, S.; Heng, H.; Krawetz, A., and Benham, C. From DNA structure to gene expression: Mediators of nuclear compartmentalization and dynamics.* ***Journal of Chromosome Research****. 2003.*
- *Bollati Fogolin, M.; Irani, N.; Beccaria, A. J.; Schulz, C.; van den Heuvel, J.; Elias, C. B.; Carpentier, E.; Durocher, Y.; Bisson, L.; Etcheverrigaray, M.; Kratje, R. B.; Wirth, M.; Kamen, A., and Wagner, R. Impact of the expression of yeast pyruvate carboxylase on the productivity of different animal host cell lines. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers. 2003.*
- *Brigelius-Flohé, R.; Maiorino, M.; Ursini, F., and Flohé, L. Selenium - an Antioxidant? In: (Cadenas, E., Packer, L., editors.) Handbook of Antioxidant. 2.ed. New York: Marcel Dekker Inc.; 2003.*
- *Bruns, K.; Fossen, T.; Wray, V.; Henklein, P.; Tessmer, U., and Schubert, U. Structural characterization of the HIV-1 Vpr N-terminus: Evidence of cis/trans proline isomerism.* ***Journal of Biological Chemistry****. 2003.*

- *Buttó, S.; Fiorelli, V.; Tripiciano, A.; Ruiz-Alverez, M. J.; Scoglio, A.; Ensoli, F.; Ciccozzi, M.; Collacchi, B.; Sabbatucci, M.; Cafaro, A.; Guzmán, C. A.; Borsetti, A.; Caputo, A.; Vardas, E.; Colvin, M.; Lukwija, M.; Rezza, G.; Ensoli, B., and and the Tat Multicentric Study Group. Sequence conservation and antibody cross-recognition of the Clade B HIV-1 Tat protein vaccine candidate in HIV-1-infected Italian, Ugandan and South African individuals.* ***Journal of Infectious Diseases****. 2003.*
- *Cesari, F.; Rennekampff, V.; Vintersten, K.; Vuong, L.; Bode, J.; Seibler, J.; Wiebel, F. F., and Nordheim, A. Site-directed mutagenesis of the murine Elk-1 locus by RMCE.* ***Genesis.*** *2003.*
- *Deiters, U.; Barsig, J.; Tawil, B., and Mühlradt, P. F. The macrophage activating lipopeptide MALP-2 accelerates wound healing in diabetic mice.* ***Journal of Investigative Dermatology****. 2003.*
- *Deiters, U.; Gumenschneider, M.; Galanos, C., and Mühlradt, P. F. TLR2/6-mediated stimulation by the lipopeptide MALP-2 induces LPS-cross tolerance in mice, resulting in protection from TNF – but in only partial protection from lethal LPS doses.* ***Infection and Immunity****. 2003; 71.*
- *Denecke, J.; Kranz, C.; Nimtz, M.; Conradt, H. S.; Brune, T.; Kienz, T.; Harms, E.; Heimpel, H., and Marquardt, T. Charakterization of the N-glycosylation phenotype of erythrocyte membrane proteins in congenitaldyserythropoetic anemia type II (CDAII/HEMPAS).* ***Blood****. 2003.*
- *Dietrich, G.; Spreng, S.; Favre, D.; Viret, J.-F., and Guzmán, C. A. Delivery of cancer DNA vaccines by live attenuated bacteria.* ***Enhancer-Immunotherapy of Cancer****. 2003.*
- *Ding, H.; Griesel, C. H.; Conradt, H.; Nimtz, M.; Weich, H. A., and Jäger, V. Molecular cloningexpression, purification and characterisation of soluble human interleukin-3 (IL-3) with a baculovirus-insect cell expression system.* ***Protein Expression and Purification****. 2003.*
- *Düber, S.; Engel, H.; Rolink, A.; Kretschmer, K., and Weiss, S. Germline transcripts of immunoglobulin light chain variable regions are structurally divers and differentially expressed.* ***Molecular Immunology****. 2003.*
- *Ebensen, T.; Link, C., and Guzmán, C. A. Classical bacterial vaccines. In: (Kaufman, S. H. E., editor). Novel Vaccination Strategies. Weinheim, Germany: Wiley-VCH Verlag GmbH; 2003.*
- *Ehlers, S.; Lauber, J.; Buer, J., and Lehmann, J. Measuring immune responses. In: (Kaufmann, S. H. E., Kabelitz, D., editors). Immunology of infection/Methods in Microbiology. 2nd Edition ed. San Diego: Academic Press; 2003; 32.*
- *Eubel, H.; Jänsch, L., and Braun, H. P. New insights into the respiratory chain of plant mitochondria: supercomplexes and a unique composition of complex II.* ***Plant Physiology****. 2003.*
- *Fargali, S.; Barthild, M.; Rohde, M.; Majore, I., and Jäger, V. In vitro cultivation of rabbit mesenchymal stromal cells on 3D bioresorbable calcium phosphate scaffolds for the generation of bone tissue implants. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers; 2003.*
- *Focke, M.; Gieringer, E.; Schwan, S.; Jänsch, L.; Binder, S., and Braun, H. P. Fatty acid biosynthesis in mitochondria of grasses: malonlyl-CoA is integrated by a mitochondrial-localized acetyl-CoA carboxylase.* ***Plant Physiology****. 2003.*
- *Franke, R.; Doll, C.; Wray, V., and Eichler, J. Solid-phase synthesis of structurally diverse scaffolded peptides for the mimicry of discontinuous protein binding sites.* ***Protein Peptide Letters.*** *2003.*
- *Gerth, K.; Pradella, S.; Perlova, O.; Beyer, S., and Müller, R. Myxobacteria: Proficient producers of novel natural products with various biological activities past and future biotechnological aspects with the focus on the genus Sorangium.* ***Journal of Biotechnology****. 2003.*
- *Grenklo, S.; Geese, M.; Lindberg, U.; Wehland, J.; Karlsson, R., and Sechi, A. S. Critical role for profilin:actin in the intracellular motility of* Listeria monocytogenes. ***EMBO Reports****. 2003.*
- *Guido, D.; Spreng, S.; Favre, D.; Viret, J.-F., and Guzmán, C. A. Live Attenuated bacteria as vectors to deliver plasmid DNA vaccines.* ***Current Opinion in Molecular Therapeutics****. 2003.*
- *Heesemann, J.; Heinz, D. W.; Rüssmann, H.; Wehland, J.; Goebel, W., and Kuhn, M. Lektionen aus der Bakterienwelt: Ausnutzung von Wirtszellprozessen durch pathogene Mikroben.* ***BioSpektrum****. 2003.*
- *Helloin, E.; Jänsch, L., and Phan-Thanh, L. Carbon starvation survival of* Listeria monocytogenes *in planktonic state and in biofilm: a proteomic study.* ***Proteomics****. 2003.*
- *Heng, H. H. Q.; Goetze, S.; Ye, C. J.; Lu, W.; Liu, G.; Bremer, S.; Hughes, M.; Bode, J., and Krawetz, S. A. Dynamic features of scaffold/matrix attached regions (S/MARs) in anchoring chromatin loops.* ***Journal of Cell Science.*** *2003.*
- *Kröger, A.; Dalügge, A.; Kirchhoff, S., and Hauser, H. IRF_1 reverts the transformed phenotype of oncogenically transformed cells* in vitro *and* in vivo. ***Oncogene****. 2003.*
- *Kuhlmeier, D.; Rodda, E.; Kolarik, L. O.; Furlong, D. N., and Bilitewski, U. Application of atomic force microscopy and grating coupler for the characterization of biosensor surfaces.* ***Biosensors and Bioelectronics****. 2003.*
- *Leibold, T.; Sasse, F.; Reichenbach, H., and HöfleG. Cyrmenins, novel antifungal peptides containing a nitrogen-linked ß-methoxyacrylate pharmacophore: Isolation and structure elucidation.* ***European Journal of Organic Chemistry****. 2003.*
- *Ma, H. and Zeng, A.-P. Phylogenetic comparision of metabolic capacities of organisms at genome level.* ***Molecular Phylogenetics and Evolution****. 2003.*
- *Paschen, A.; Schadendorf, P., and Weiss, S. Bacteria as vector for gene therapy of cancer. In: (Templeton, N. S., Lasic, D. D., editors). Gene Therapy: Therapeutic mechanisms and strategies. New York, Basel: Marcel Dekker; 2003.*
- *Ramnath, M.; Rechinger, K. B.; Jänsch, L.; Hastings, J. W.; Knochel, S., and Gravesen, A. The development of a* Listeria monocytogenes *EGDe proteome reference map and comparison with food isolates.* ***Applied and Environmental Microbiology****. 2003.*
- *Rasmussen, U.; Schreiber, V.; Schultz, H.; Mischler, F., and Schughart, K. Tumor cell targeting by phage displayed peptides.* ***Cancer Gene Therapy****. 2003.*
- *Sasse, F.; Leibold, T.; Kunze, B.; Höfle, G., and Reichenbach, H. Cyrmenins, new ß-Methoxyacrylate of the respiratory chain isolated from Myxobacteria.* ***European Journal of Organic Chemistry****. 2003.*
- *Satyanarayana, A.; Wiemann, S. U.; Buer, J.; Lauber, J.; Wüstefeld, T.; Blasco, M.; Manns, M. P., and Rudolph, K. L. Telomere shortening impairs organ regeneration by inhibition of cell cycle re-entry in a sub-population of cells with critical short telomeres.* ***EMBO Journal****. 2003.*
- *Schubert, W.-D. and Heinz, D. W. Structural aspects of adhesion and Invasion of host cells by the human pathogen* Listeria monocytogenes. ***ChemBioChem****. 2003.*
- *Schulze, K. and Guzmán, C. A. Identification of the domains of the fibronectin-binding protein I of* Streptococcus pyogenes *responsible for adjuvanticity.* ***FEMS Immunology and Medical Microbiology****. 2003.*
- *Söker, U.; Kunze, B.; Reichenbach, H., and HöfleG. Dawenol, a new polyene metabolite from the myxobacterium* Stigmatella aurantiaca. ***Zeitschrift für Naturforschung****. 2003.*
- *Toppel, A.; Rasmussen, M.; Rohde, M.; Medina, E., and Chhatwal, G. S. Contribution of protein GRAB to bacterial virulence in a murine skin model of group A streptococcal infection.* ***Journal of Infectious Diseases****. 2003.*

- *Viret, J.-F.; Moser, C.; Rharbaoui, F.; Metcalfe, I. C., and Guzmán, C. A. Virosomal technology and mucosal adjuvants. In: (Kaufmann, S. H. E., editor). Novel Vaccination Strategies. Weinheim, Germany: Wiley-VCH Verlag GmbH; 2003.*

- *Wehmhöner, D.; Haussler, S.; Tümmler, B.; Jänsch, L.; Wehland, J., and Steinmetz. I. Inter - and intraclonal diversity of* Pseudomonas aeruginosa *proteome manifests within the secretome.* ***Journal of Bacteriology****. 2003.*

- *Weinig, S.; Hecht, H. J.; Mahmud, T., and Müller, R. Myxothiazol and the melithiazol biosynthesis in myxobacteria: Novel insights into hybrid PKS/NRPS systems and evidence for a new subclass of SAM-dependent methyl transferase from* Mellittangium lichenicola *Me 146.* ***Chemistry and Biology****. 2003.*

- *Weinig, S.; Mahmud, T., and MüllerR. Markerless in frame deletions and point mutations in the myxothiazol biosynthetic genes provide evidence for a delicate megasynthetase with a superfluous nonribosomal peptide synthetase domain.* ***Chemistry and Biology****. 2003.*

- *Weiss, S. Transfer of eukaryotic expression plasmids to mammalian hosts by attenuated* Salmonella sp. ***International Journal of Medicine and Microbiology****. 2003.*

- *Weiss, S. and Chakraborty, T. Transfer of eukaryotic expression plasminds to mammalian host cells by Gramm-negative bacteria. In: (Goebel, W., Dietrich, G., editors). Vaccines: Delivery systems Chapt.: Horizon Scientific Press; 2003; 13.*

- *Wiethe, C.; Dittmar, K. E. J.; Doan, T.; Lindenmaier, W., and Tindle, R. Privision of 4-1BBL enhances effector and memory CTL responses generated by immunisation with dendritic cells expressing a human tumour associated antigen.* ***Journal of Immunology****. 2003.*

- *Wirth, D. and Hauser, H. Flp-mediated integration of expression cassettes into FRT tagged chromosomal loci. In: (Balbas, P., Lorence, A., edoitors). Recombinant Protein Protocols: Humana Press Inc., 2003.*

- *Zander, K.; Sherman, M. P.; Tessmer, U.; Bruns, K.; Wray, V.; Prechter, A. T.; Schubert, E.; Henklein, P.; Neidleman, J., and Greene, W. C. Schubert U. Functional association of cyclophilin A with HIV-1 Vpr.* ***Journal of Biological Chemistry****. 2003.*

- *Zielinski, M.; Kahl, S.; Hecht, H.-J., and Hofer, B. Pinpointing biphenyl dioxygenase residues that are crucial for substrate interaction.* ***Journal of Bacteriology****. 2003.*

Comparative Genome Research

- *Bi, J.-X.; Buhr, P.; Zeng, A. P., and Wirth, M. Human c-fos promoter mediates high-level, inducible expression in various mammalian cell lines.* ***Biotechnology and Bioengineering****. 2003;* ***81****: 848-854.*

- *Bialek, K.; Swistowski, A., and Frank, R. Epitope-targeted proteome analysis: Towards a large-scale automated protein-protein-interaction mapping utilizing synthetic peptide arrays.* ***Analytical and Bioanalytical Chemistry****. 2003;* ***376(7)****:1006-1013.*

- *Budde, H.; Flohé, L.; Hecht, H.-J.; Hofmann, B.; Stehr, M.; Wissing, J., and Lünsdorf, H. Kinetics and redox-sensitive oligomerisation reveal negative subunit cooperativity in trypadoxin peroxidase of* Trypanosoma brucei brucei. ***Biological Chemistry****. 2003;* ***384****:619-633.*

- *Eichler, J. Kombinatorische Chemie. Konzepte und Strategien. Teubner Studienbücher Chemie. Stuttgart: Teubner, B. G.; 2003.*

- *Eichler, J.; Hirsch, T., and Overwin, H. Synthetic mimicry of con-formationally defind protein binding sites: hYAP-WW domain. In: (Benedetti, E. Cedone C., editors). Peptides 2002. Napoli, Italy; 2003; pp. 740-741.*

- *Kim, E.-J.; Sabra, W., and Zeng, A.-P. Iron deficiency leads to inhibition of oxygen transfer and enhanced formation of virulence factors in* Pseudomonas aeruginosa *cultures.* ***Microbiology.*** *2003;* ***149****:2627-2634.*

- *Krull, M.; Voss, N.; Choi, C.; Pistor, S.; Potapov, A., and Wingender, E. TRANSPATH®: an integrated database on signal transduction and a tool for array analysis.* ***Nucleic Acids Research****. 2003;* ***31****:97-100.*

- *Kuper, J.; Winking, J.; Hecht, H.-J.; Mendel, R. R., and Schwarz, G. The active site of molybdenum cofactorbiosynthetic protein domain Cnx 1 G.* ***Archives of Biochemistry and Biophysics****. 2003;* ***41****:36-46.*

- *Ma, H. and Zeng, A.-P. Reconstruction of metabolic networks from genome data and analysis of their global structure for various organisms.* ***Bioinformatics****. 2003;* ***19****:270-277.*

- *Ma, H. and Zeng, A. P. The connectivity structure, giant strong component and centrality of metabolic networks.* ***Bioinformatics****. 2003;* ***19****:1423-1430.*

- *Matys, V.; Fricke, E.; Geffers, R.; Gößling, E.; Haubrock, M.; Hehl, R.; Hornischer, K.; Karas, D.; Kel, A. E.; Kel-Margoulis, E. V.; Kloos, D. U.; Land, S.; Lewicki-Potapov, B.; Michael, H.; Münch, R.; Reuter, I.; Rotert, S.; Saxel, H.; Scheer, M.; Thiele, S., and Wingender, E. TRANSFAC®: transcriptional regulation, from patterns to profiles.* ***Nucleic Acids Research****. 2003;* ***31****:374-378.*

- *Moser, J.; Frere, F.; Heinz, D. W.; Jahn, D., and Schubert, W.-D. Die tRNA-abhängige Tetrapyrrol-Biosynthese.* ***BioSpektrum****. 2003;* ***9****:133-137.*

- *Münch, R. ; Hiller, K.; Barg, H.; Heldt, D.; Linz, S.; Wingender, E., and Jahn, D. PRODORIC: prokaryotic datbase of gene regulation.* ***Nucleic Acids Research****. 2003;* ***31****:266-269.*

- *Qiao, J.-J.; Yuan, Y.-J.; Zhaol, H.; Wu, J.-C.; Zeng, A.-P. Apoptotic cell death in suspension cultures of* Taxus cuspidata *co-treated with sylicylic acid and hydrogen peroxide.* ***Biotechnology Letters.*** *2003;* ***25****:387-390.*

- *Sabra, W.; Lünsdorf, H., and Zeng, A.-P. Alterations in the formation of lipopolysaccharide and membrane vesicle on the surface of* Pseudomonas aeruginosa *PAO1 under oxidative stress conditions.* ***Microbiology.*** *2003;* ***149****:2789-2795.*

- *Shelest, E.; Kel, A. E.; Gößling, E., and Wingender, E. Prediction of potential C/EBP/NF-kappaB composite elements using the matrix-based search methods.* ***In Silico Biology****. 2003;* ***3(1-2)****:71-79.*

- *Sobrado, V.; Montemartini-Kalisz, M.; Kalisz, H.; De La Fuente, M. C.; Hecht, H.-J., and Nowicki, C. Involvement of conserved asparagine and arginine residues from the n-terminal region in the catalytic mechanism of rat liver and* Trypanosoma cruzi *tyrosine aminotransferases.* ***Protein Sciences****. 2003;* ***12****:1039-1050.*

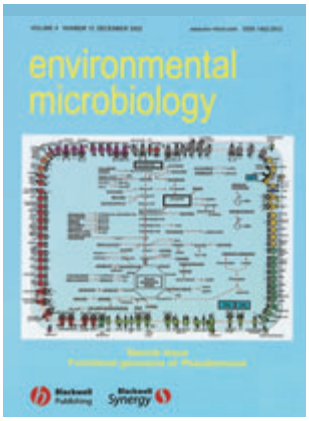
- *Sun, J.; van den Heuvel, J.; Soucaille, P.; Qu, Y., and Zeng, A. P. Comparative genomic analysis of dha regulon and related genes for anaerobic glycerol metabolism in microorganisms.* ***Biotechnology Progress****. 2003;* ***19****:263-272.*

- *Wang, W.; Sun, J.; Hartlep, M.; Deckwer, W.-D., and Zeng, A.-P. Combined use of proteomics analysis and enzyme activity assay for metabolic pathway analysis of glycerol fermentation by* Klebsiella pneumoniae. ***Biotechnology and Bioengineering****. 2003;* ***83****:525-536.*

- *Zander, N.; Gerhardt, J., and Frank, R. Polystyrylsulfonyl-3-nitro-1H-1,2,4-triazolide-resin: a new solid-supported reagent for the esterification of amino acids.* ***Tetrahedron Letters****. 2003;* ***44(35)****:6557-6560.*

Comparative Genome Research – in press

- *Franke, D.; Lorbach, V.; Dose, C.; Essner, S.; Halfar, M.; Thömmes, J.; Müller, R.; Takors, R.; Sprenger, G. A., and Müller, M. (5S,6S)-Dihydroxy-cyclohexa-1,3-dienecarboxylic acid: microbial access with engineered cells of* Escherichia coli *and applicability as starting material in natural product synthesis.* ***Chemistry - A European Journal****. 2003.*
- *Jahn, D.; Moser, J.; Schubert, W.-D., and Heinz, D. W. Transfer RNA-dependent aminolevulinic acid formation: structure and function of glutamyl-tRNA synthase, reductase and glutamate-1-semialdehyde-2,1-aminomutase. In: Chlorophylls. Boca Raton: CRC Press; 2003.*
- *Kauer, G. and Blöcker, H. Applying signal theory to the analysis of biomolecules.* ***Bioinformatics****. 2003.*
- *Ma, H., and Zeng, A.-P. Phylogenetic comparision of metabolic capacities of organisms at genome level.* ***Molecular Phylogenetics and Evolution.*** *2003.*
- *Schubert, W.-D.; Moser J.; Heinz, D. W., and Jahn, D. Structure an function of glutamyl-tRNA reductases, the first enzyme of tetrapyrrole biosynthesis in plants and bacteria.* ***Photosynthesis Research****. 2003.*
- *Wang, W.; Sun, J.; Nimtz, M.; Zeng, A.-P., and Deckwer, W.-D. Protein identification from 2-dimensional gel electrophoresis analysis of* Klebsiella pneumoniae *by combined use of mass spectrometry data and unannotated genome sequences.* ***Proteome Science****. 2003.*
- *Zander, N. and Frank, R. The use of polystyrylsulfonyl chloride resin as a solid supported condensation reagent for the formation of esters: Synthesis of -Butyl-ß-{2-[N-ethyl-4-(4-nitro-phenylazo)-anilino]-ethyl}-N--(9-fluorenyl-methoxy-carbonyl)-L-aspartate.* ***Organic Synthesis****. 2003.*
- *Zeng, A.-P. and Bi, J. Cell culture kinetics and modelling. In: (Ozturk, S. S., Hu, W.-S., editors). Cell Culture Technology for Pharmaceutical and Cellular Therapies; 2003; p. Chapter 16.*



- *Cover picture of the journal Environmental Microbiology, Vol. 4 (12), 2002, on the occasion of the publication of the article Nelson, K. E.; Weinel, C.; Paulsen, I. T.; Dodson, R. J.; Hilbert; Martins dos Santos, V.; Fouts, D. Gill S. R.; Pop, M.; Holmes, M.; Khouri, H.; Hance, I.; Chris Lee, P.; Holtzapple, E.; Scanlan, D.; Tran, K.; Deboy, R.; Moazzez, A.; Brinkac, L.; Beanan, M.; Daugherty, S.; Kolonay, J.; Madupu, R.; Nelson, W.; White, O.; Utterback, T.; Rizzo, M.; Lee, K.; Kosack, D.; Moestl, D.; Wedler, H.; Lauber, J.; Hoheisel, J.; Strätz, M.; Heim, S.; Kiewitz, C.; Eisen, J.; Timmis, K. N.; Duesterhoft, A.; Tümmler, B., and Fraser, C. M. Complete genome sequence and comparative analysis of the metabolically versatile* Pseudomonas putida *KT2440.* ***Environmental Microbiology****. 2002;* ***4****: 799-808. The permission of Blackwell Publishing is gratefully acknowledged.*

Sustainable Use of Landscapes

- *Abraham, W.-R. and Hesse, C. Isotope fractionations in the biosynthesis of cell compounds by different fungi: A basis for environmental carbon flux studies.* ***FEMS Microbiology Ecology****. 2003;* ***46****:121-128.*
- *Abraham. W.-R.; Lünsdorf, H.; Strömpl, C.; Nogales, B.; Moore, E. R. B., and Timmis, K. N. Microbial communities in composite biofilms participating in the degradation of PCB.* ***Air Soil Pollution: Focus: Bioremediation****. 2003;* ***3****:57-64.*
- *Abraham, W.-R. and Wenderoth, D. F. Schicksal fakultativ pathogener Mikroorganismen während und nach dem Sommerhochwasser an Elbe und Mulde. In: (Geller, W.; Ockenfeld, K.; Böhme, M.; Voigt, M., editors). Schadstoffbelastung im Mulde- und Elbeeinzugsgebiet nach dem Augusthochwasser 2002, Magdeburg. 2003; pp. 6-9.*
- *Allgaier, M.; Uphoff, H.; Felske, A., and Wagner-Döber, I. Aerobic anoxygenic photosynthesis in* Roseobacter *clade from diverse marine habitats.* ***Applied and Environmental Microbiology****. 2003;* ***69(9)****:5051-5059.*
- *Bertini, I.; Provenzani, A.; Viezzoli, M. S.; Pieper, D. H., and Timmis, K. N. NMR spectroscopy as a tool to investigate the degradation of aromatic compounds by a* Pseudomonas putida *strain.* ***Magnetic Resonance in Chemistry****. 2003;* ***41****:615-621.*
- *Brettar, I.; Christen, R., and Höfle, M.G.* Idiomarina baltica, *sp. nov., a marine bacterium with high temperature optimum isolated from surface water of the Central Baltic Sea.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:407-413.*
- *Brummer, I. H.; Felske, A., and Wagner-Döbler, I. Diversity and seasonal variability of beta-Proteobacteria in biofilms of polluted rivers: analysis by temperature gradient gel electrophoresis and cloning.* ***Applied and Environmental Microbiology****. 2003;* ***69(8)****:4463-73.*
- *Demnerova, K.; Stiborova, H.; Leigh, M. B.; Pieper, D. H.; Pazlarova, J.; Brenner, V., and Macek, T. Bacteria degrading PCBs and CBs isolated from long-term PCB contaminated soil.* ***Water, Air and Soil Pollution: Focus****. 2003;* ***3****:47-55.*
- *Hahn, M. W.; Lünsdorf, H.; Wu, Q.; Schauer, M.; Höfle, M.G.; Boenigk, J., and Stadler, P. Isolation of novel ultramicrobacteria classified as Actinobacteria from five freshwater habitats in Europe and Asia.* ***Applied and Environmental Microbiology****. 2003;* ***69****:1442-1451.*
- *Kahl, S. and Hofer, B. A genetic system for the rapid isolation of aromatic-ring-hydroylating dioxygenase activities.* ***Microbiology****. 2003;* ***149****:1475-1481.*
- *Kämpfer, P.; Dreyer, U.; Neef, A.; Dott, W., and Busse, H.-J.* Chryseobacterium defluvii *sp. nov., isolated from wastewater.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:93-97.*
- *Mauclaire, L.; Thullner, M.; Pelz, O.; Abraham, W.-R. and Zeyer, J. Assimilation of toluene carbon along a bacteria–protist food chain determined by 13C-enrichment of biomarker fatty acids.* ***Journal of Microbiological Methods****. 2003;* ***55****:635-649.*
- *Lucas, F.; Bertru, G., and Höfle, M.G. Characterization of free-living and attached bacteria in sediments colonized by* Hediste (Nereis) diversicolor. ***Aquatic Microbial Ecology****. 2003;* ***32****:165-174.*
- *McKay, D.; Prucha, W.; Reineke, K.; Timmis, K. N., and Pieper, D. H. Substrate specificity and expression of three 2,3-dihydroxybiphenyl 1,2dioxygenases from* Rhodococcus globerulus *strain P6.* ***Journal of Bacteriology****. 2003;* ***185****:2944-2951.*
- *Perez-Pantoja, D. T.; Ledger, D.; Pieper, D., and Gonzalez, B. Efficient turnover of chlorocatechols is essential for growth of* Ralstonia eutropha *JMP134(pJP4) in 3-chlorobenzoic acid.* ***Journal of Bacteriology****. 2003;* ***185****:1534-1542.*
- *Strömpl, C.; Hold, G. N.; Lünsdorf, H.; Graham, J.; Gallacher, S.; Abraham, W.-R.; Moore, E. R. B., and Timmis, K. N.* Oceanicaulis alexandrii *gen. nov., sp. nov., a novel stalked bacterium isolated from a culture of the dinoflagellate* Alexandrium tamarense *(Lebour) Balech.* ***International Journal of Systematic and Evolutionary Microbiology.*** *2003;* ***53****:1901-1906.*
- *Von Canstein, H. F.; Li, Y.; Leonhäuser, J.; Deckwer, W.-D., and Wagne-Döbler, I. Mikrobielle Reinigung von quecksilberhaltigen Industrieabwässern.* ***BIOspektrum****. 2003;* ***(2)****:150-152.*
- *Wagner-Döbler, I. Microbial inoculants - snake oil or panacea. In: (Singleton, I.; Milner, M. G., Head, I. M., editors). Bioremediation - A Critical Review: Horizon Press; 2003;(Chapter 10): pp. 1-31.*
- *Wagner-Döbler, I.; Rheims, H.; Felske, A.; Pukall, R., and Tindall, B.* Jannaschia helgolandensis, *gen. nov., a novel abundant member of the marine* Roseobacter *clade from the North Sea.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:731-738.*
- *Wagner-Döbler, I.; von Canstein, H. F.; Li, Y.; Leonhäuser, J., and Deckwer, W.-D. Process-integrated microbial mercury removal from wastewater of chlor-alkali electrolysis plants.* ***Engineering in Life Sciences****. 2003;* ***3(4)****:177-181.*
- *Wenderoth, D. F.; Rosenbrock, P.; Abraham, W. R.; Pieper, D. H., and Höfle, M.G. Bacterial community dynamics during biostimulation and bioaugmentation experiments aiming at chlorobenzene degradation in groundwater.* ***Microbial Ecology****. 2003;* ***46****:161-176.*
- *Witte, U.; Wenzhöfer, F.; Sommer, S.; Boetius, A.; Heinz, P.; Aberle, N.; Sand, M.; Cremer, A.; Abraham, W.-R.; Jörgensen, B. B., and Pfannkuche, O.* In situ *experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor.* ***Nature****. 2003;* ***424****:763-766.*
- *Yakimov, M. M.; Giuliano, L.; Gentile, G.; Cristafi, E. ; Chernikova, T. N.; Abraham, W.-R.; Lünsdorf, H.; Timmis, K. N., and Golyshin, P. N.* Oleispira antarctica *gen. nov., sp. nov., a new hydrocarbonoclastic marine bacterium, isolated from an antarctic coastal seawater.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:779-785.*
- *Yakimov, M. M.; Lünsdorf, H., and Golyshin, P. N.* Thermoleophilum album *and* Thermoleophilum minutum *are culturable representatives of group 2 of the Rubrobacteidae (Actinobacteria).* ***International Journal of Systematic and Evolutionary Microbiology****. 2003;* ***53****:377-380.*

Sustainable Use of Landscapes – in press

- *Barreiros, L.; Nogales, B.; Manaia, C. M.; Ferreira, A. C. S.; Pieper, D. H.; Reis, A. M., and Nunes, O. C. A novel pathway for mineralization of the thiocarbamate herbicide molinate by a defined bacterial consortium.* ***Environmental Microbiology****. 2003.*
- *Brummer, I. H. M.; Felske, A., and Wagner-Döbler, I. Diversity and seasonal variability of b-Proteobacteria in biofilms of polluted rivers analyzed by TGGE and cloning.* ***Applied and Environmental Microbiology****. 2003.*
- *Druschel, G. K.; Labrenz, M.; Thomsen-Ebert, T.; Fowle, D. A., and Banfield, J. F. Geochemical modeling of ZnS in biofilms:An example of ore depositional processes.* ***Economic Geology and the Bulletin of the Society of Economic Geologists****. 2003.*
- *Fritz, I.; Strömpl, C. and Abraham, W.-R. The phylogenetic relationships of the genera Stella, Labrys, and Angulomicrobium within the "Alphaproteobacteria", and description of* Angulomicrobium amanitiforme *sp. nov.* ***International Journal of Systematic and Evolutionary Microbiology.*** *2003.*

- *Golyshin, P. N.; Harayama, S.; Timmis, K. N., and Yakimov, M. M. Family Alcanivoraceae. In: Bergey's Manual of Systematic Bacteriology. New York: Springer; 2003.*
- *Hallworth, J. E.; Prior, B. A.; Nomura, Y., and Timmis, K. N. Compatible solutes protect against chaotrope-(ethanol-) induced, non-osmotic water stress.* ***Applied and Environmental Microbiology****. 2003.*
- *Heim, S.; Heuer, H.; Regenhardt, D.; Ferrer, M.; Nimtz, M., and Timmis, K. N. Proteome reference map of* Pseudomonas putida *strain KT2440 for gene profiling analysis: comparative whole genome analysis of iron deprivation in KT2440 and* P. aeruginosa *strain PAO1.* ***Environmental Microbiology****. 2003.*
- *Höfle, M.G. Genotyping of bacterial isolates from the environment using low-molecular-weight RNA fingerprints. In: (Akkermans, A. D. L., Elsas, J. D., de Bruijn, F. J., editors). Molecular Microbial Ecology Manual. 2nd ed., Dordrecht, Netherlands: Kluwer Academic Publishers. 2003.*
- *Junca H. and Pieper, D. H. Amplified functional DNA restriction analysis to determine catechol 2,3 dioxygenase gene diversity in soil bacteria.* ***Journal of Microbiological Methods****. 2003.*
- *Labrenz, M. and Hirsch, P. The Genus Antarctobacter. In: Bergey's Manual of Systematic Bacteriology: Bergey's Manual Trust; 2003.*
- *Labrenz, M. and Hirsch, P. The Genus Roseovarius. In: Bergey's Manual of Systematic Bacteriology: Bergey's Manual Trust; 2003.*
- *Labrenz, M. and Hirsch, P. The Genus Staleya. In: Bergey's Manual of Systematic Bacteriology: Bergey's Manual Trust; 2003.*
- *Labrenz, M.; Lawson, P. A.; Tindall, B. J.; Collins, M. D., and Hirsch,* P. Saccharospirillum impatiens *gen. nov., sp. nov., a novel gamma-Proteobacterium isolated from hypersaline Ekho Lake (East Antarctica).* ***International Journal of Systematic and Evolutionary Microbiology****. 2003; 53.*
- *Nikitin, D.; Strömpl, C.; Oranskaya, M. S. and Abraham, W.-R. Phylogeny of the ring-forming bacterium* Arcicella aquatica *gen. et sp. nov. (ex Nikitin et al. 1994) from a freshwater neuston.* ***International Journal of Systematic and Evolutionary Microbiology.*** *2003.*
- *Pieper, D. H. and Reineke, W. Degradation of chloroaromatics by Pseudomona(d)s. The Pseudomonads Vol. III. Biosynthesis of Macromolecules and Molecular Metabolism. New York: Kluwer Academic/Plenum Publishers; 2003.*
- *Prucha, M.; McKay, D.; Timmis, K. N., and Pieper, D. H. Substrate specificity and expression of three 2,3-dihydroxybiphenyl 1,2-dioxygenases from* Rhodococcus globerulus *strain P6.* ***Journal of Bacteriology****. 2003.*
- *Rapp, P. and Gabriel-Jürgens, L. H. E. Degradation of alkanes, highly chlorinated benzenes and production of biosurfactans by a psychotrophic* Rhodococcus *sp. Genetic characterization of its chlorobenzene dioxygenase.* ***Microbiology****. 2003.*
- *Torres, K. N.; Jaenecke, S.; Timmis, K. N.; Garcia, J., and Diaz, E. A dual lethalsystem to enhance predictability of recombiant microorganisms.* ***Environmental Microbiology****. 2003.*
- *Vancanneyt, M.; Segers, P.; Abraham, W.-R., and de Vos, P. Genus Brevundimonas Segers, Vancanneyt, Pot, Torck, Hoste Dewettinck, Falsen, Kersters, de Vos 1994, 507VP emend. Abraham, Strömpl, Meyer, Lindholst, Moore, Christ, Vancanneyt, Tindall, Bennasar, Smit, Tesar 1999, 1070VP. Bergey's Manual of Systematic Bacteriology; 2003; 2(George M. Garrity).*
- *Yakimov, M. M.; Giuliano, L.; Denaro, R.; Cristafi, E.; Chernikova, T.; Abraham, W.-R.; Luensdorf, H. ; Timmis, K. N., and Golyshin, P. N.* Thalassolituus oleivorans, *gen. nov. sp. nov., and new marine bacterium confined to the utilization of hydrocarbons.* ***International Journal of Systematic and Evolutionary Microbiology****. 2003.*

Technological Platforms

- *Bringmann, G.; Mühlbacher, J.; Messer, K.; Deyer, M.; Ebel, R.; Nugroho, B. W.; Wray, V., and Proksch. P. Cyclorocaglamide, the first bridged cyclopentatetrahydrobenzofuran and a relatted „open chain" rocaglamide derivative from* Alaia oligophylla. ***Journal of Natural Products****. 2003;* ***66****:80-85.*
- *Clauss, M.; Pipp, F.; Issbrücker, K.; Weich, H.; Heil, M., and Schaper, W. Dissection of monocyte and endothelial activities by using VEGF-receptor specific ligands.* ***Advances in Experimental Medicine and Biology****. 2003;* ***522****:75-82.*
- *Häußler, S.; Rohde, M.; von Neuhoff, N.; Nimtz, M., and Steinmetz, I. Structural and functional cellular changes induced by* Burholderia pseudomallei *rhamnolipid.* ***Infection and Immunity****. 2003;* ***71****:2970-2975.*
- *Lin, W.; Brauers, G.; Ebel, R.; Wray, V.; Berg, A.; Sudarsono, and Proksch, P. Novel chromone derivates from the fungus* Aspergillus versicolor *isolated from the marine sponge* Xestospongia exiqua. ***Journal of Natural Products****. 2003;* ***66****:57-61.*
- *Marxen, J. C.; Nimtz, M.; Becker, W., and Mann, K. The major soluble 19,6kda protein of the organic shell matrix of the freshwater snail* Biomphalaria glabrata *is an N-glycosylated dermapontin.* ***Biochimica et Biophysica Acta****. 2003;* ***1650****:92-98.*
- *Münzenberger, B.; Hammer, E.; Wray, V.; Shauer, F.; Schmidt, J., and Strack, D. Detoxification of ferulic acid by ectomycorrhizal fungi.* ***Mycorrhiza****. 2003;* ***13****:117-121.*
- *Pipp, F.; Heil, M.; Issbrücker, K.; Ziegelhoeffer, T.; Martin, S.; van den Heuvel, J.; Weich, H.; Fernandez, B.; Colomb, G.; Caremeliet, P.; Schaper, W., and Claus, M. The VEGFR-1 selective VEGF-homologue PIGF is arteriogenic: evidence for a monocyte mediated mechanism.* ***Circulation Research****. 2003;* ***92****:378-385.*
- *Pollmann, K.; Wray, V.; Hecht, H.-J., and Pieper, D. H. Rational engineering of the regioselectivity of TecA tetrachlorobenzene dioxygenase for the transformation of chlorinated toluenes.* ***Microbiology****. 2003;* ***149****:903-913.*
- *Proksch, P.; Ebel, R.; Erada, R. A.; Schupp, P.; Lin, W. H.; Sudarsono; Wray, V., and Steube, K. Detection of pharmacologically active natural products using ecology. Selected examples from Indopacific marine invertebrates and sponge-derived fungi.* ***Pure and Applied Chemistry****. 2003;* ***75****:337-346.*
- *Schultz, A.; Laschat, S.; Diele, S., and Nimtz, M. Tetraphenylethene-derived columnar crystals and their oxidative photocyclization.* ***European Journal of Organic Chemistry****. 2003; 2829-2839.*
- *Schupp, P.; Poehner, T.; Erada, R.; Ebel, R.; Wray, V., and Proksch, P. Eudistomins W and X, two new ß-carbolines from the Micronesian Tunicate* Eudistoma viride. ***Journal of Natural Products****. 2003;* ***66****:272-275.*
- *Wang, C.-Y.; Wang, B.-G.; Wiroyowidago, S.; Wray, V.; van Soest, R.; Steube, K. G.; Guan, H.-S.; Proksch, P., and Ebel, R. Melophins C-O, thirteen novel teramic acids from the marine sponge* Melophlus sarassinorum. ***Journal of Natural Products****. 2003;* ***66****:51-56.*
- *Zogai, X.; Bokranz, W.; Nimtz, M., and Römling, U. Cellulose and curli fimbriae producing enterobacteriaceae isolated from the human gastrointestinal tract.* ***Infection and Immunity****. 2003;* ***71****:4151-4158.*
- *Zorn, H.; Langhoff, S.; Scheibner, M.; Nimtz, M., and Berger, R. G. Cleavage of ß,ß-carotene to flavourcompounds by* Lepista irina *versatile peroxidase.* ***Biochemical Journal****. 2003;* ***384****:1049-1056.*

Technological Platforms – in press

- *Ding, H.; Griesel, C. H.; Conradt, H.; Nimtz, M.; Weich, H. A., and Jäger, V. Molecular cloningexpression, purification and characterisation of soluble human interleukin-3 (IL-3) with a baculovirus-insect cell expression system.* ***Protein Expression and Purification****. 2003.*

- *Eming, S. A.; Lauer, G.; Cole, M.; Jurk, S.; Christ, H.; Hornig, C.; Krieg, T., and Weich, H. Increased levels of the soluble variant of the vascular endothelial growt factor receptor VEGFR-1 (sflt-19) are associated with poor prognosis in wound healing.* ***Lancet****. 2003.*

- *Heil, M.; Mittnacht-Krauss, R.; Issbrücker, K.; van den Heuvel, J.; Dehio, C.; Schaper, W.; Clauss, M., and Weich, H. A. An engineered heparin-binding form of VEGF-E: biological effects* in vitro *and mobilisation of precursor cells.* ***Angiogenesis****. 2003.*

- *Satyanarayana, A.; Wiemann, S. U.; Buer, J.; Lauber, J.; Wüstefeld, T.; Blasco, M.; Manns, M. P., and Rudolph, K. L. Telomere shortening impairs organ regeneration by inhibition of cell cycle re- entry in a sub-population of cells with critical short telomeres.* ***EMBO Journal****. 2003.*

Biotech Facilities

- *Elias, C. B.; Carpentier, E.; Durocher, Y.; Bisson, L.; Wagner, R., and Kamen, A. Improving glucose and glutamine metabolism of human HEK 293 and* Trichoplusia ni *insect cells engineered to express a cytosolic pyruvate carboxylase enzyme.* ***Biotechnology Progress****. 2003;* ***19****:90-97.*

- *Hesse, F.; Ebel, M.; Konisch, N.; Sterlinski, R.; Kessler, W., and Wagner, R. Comparison of a production process in a membrane-aerated stirred tank and up to 1000 L airlift bioreactors using BHK-21 cells and chemically defined protein-free medium.* ***Biotechnology Progress****. 2003;* ***19****:833-843.*

- *Horn, H.; Cordes, C.; Krull, R.; Hempel, D. C.; Jahn, D., and Rinas, U. Einfluss der Pelletmorphologie von* Aspergillus niger *auf Stoffumsatz und Produktbildung - Experimentelle Untersuchungen und Modellierung.* ***Chemie Ingenieur Technik.*** *2003;* ***75****:1070-1071.*

- *Müller, C.; Richter, S., and Rinas, U. Kinetic control preferential heterodimer formation of platelet-derived growth factor from unfolded A- and B-chains.* ***Journal of Biological Chemistry****. 2003;* ***278****:18330-18335.*

- *Priesner, C.; Elmahouhoub, A., and Wagner, R. Immortale Hepatocyten zur Analyse leberspezifischer Funktionen.* ***Bioforum****. 2003;* ***1-2****:35-37.*

- *Wang, W.; Sun, J.; Hartlep, M.; Deckwer, W.-D., and Zeng, A.-P. Combined use of proteomics analysis and enzyme activity assay for metabolic pathway analysis of glycerol fermentation by* Klebsiella pneumoniae. ***Biotechnology and Bioengineering****. 2003;* ***83****:525-536.*

Biotech Facilities – in press

- *Ahmed Elsayed, A.; Piehl, G.-W.; Medronho, R. A.; Deckwer, W.-D., and Wagner, R. Use of a hydrocyclone as an efficient and easily scalable perfusion system for mammalian cell bioreactors. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers. 2003.*

- *Barthold, M.; Majore, I.; Fargali, S.; Stahl, F.; Schulz, R.; Lose, S.; Mayer, H., and Jäger, V. 3D-cultivation and characterisation of osteogenic cells for the production of highly viable bone tissue implants. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers. 2003.*

- *Bassani Molinas, M. M.; Nelving, A.; Beer, C.; Hesse, F.; Wirth, M.; Durocher, Y.; Kamen, A., and Wagner, R. Intracellular nucleotide pools for optimizing product-oriented transient tranfection of HEK293 cells in suspension. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers. 2003.*

- *Bollati Fogolin, M.; Irani, N.; Beccaria, A. J.; Schulz, C.; van den Heuvel, J.; Elias, C. B.; Carpentier, E.; Durocher, Y.; Bisson, L.; Etcheverrigaray, M.; Kratje, R. B.; Wirth, M.; Kamen, A., and Wagner, R. Impact of the expression of yeast pyruvate carboxylase on the productivity of different animal host cell lines. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers. 2003.*

- *Hesse, F.; Nelving, A., and Wagner, R. Correlation of intracellular nucleotide pools to amino acid concentrations in culture media by the application of multivariate methods. In: Animal Cell Technology Meets Genomics. Dordrecht: Kluwer Academic Publishers. 2003.*

- *Hoffmann, F. and Rinas, U. Roles of heat-shock chaperones in the production of recombinant proteins in* Escherichia coli. ***Advances in Biochemical Engineering and Biotechnology****. 2003.*

- *Hoffmann, F. and Rinas, U. Stress induced by recombiant protein production in* Escherichia coli. ***Advances in Biochemical Engineering and Biotechnology****. 2003.*

- *Hoffmann, F.; van den Heuvel, J.; Zidek, N., and Rinas, U. Minimizing inclusion body formation during recombiant protein production in* Escherichia coli *at bench and pilot plant scale.* ***Enzyme and Microbial Technology.*** *2003.*

- *Vallejo, F. and Rinas, U. Optimized procedure for renaturation of recombiant human bone morphogenetic protein-2 at high protein concentration.* ***Biotechnology and Bioengineering****. 2003.*

- *Cover picture of the journal Cellular Microbiology, Vol. 5 (5), 2003, on the occasion of the publication of the article Rohde, M.; Müller, E.; Chhatwal, G. S., and Talay, S. R. Host cell caveolae act as an entry-port for Group A streptococci.* ***Cellular Microbiology****. 2003;* ***5****:323-342. The permission of Blackwell Publishing is gratefully acknowledged.*

Richard Radloff is showing the GBF facilities to the participants of the 2003 InWEnt GBF course (le). A presentation during the workshop of Lower Saxonian Networking Project "Marine Biotechnology" in the GBF-FORUM (ce). A relaxing atmosphere near the GBF-FORUM (ri).

Photos: GBF (le), Bierstedt (ce), Radde (ri)

SCIENTIFIC REPORTS

INNOVATION REPORT

INNOVATION REPORT

Prof. Dr. Rainer Jonas | Department of Scientific Information

1. Research Financing

In 2002, the total costs of the GBF amounted to 47.8 Mio. € with more than half, 27.3 Mio. €, devoted to the programme "Infection and Immunity". Each of the other three programmes, "Comparative Genome Research", "Sustainable Use of Landscape", and "Biochemical Engineering" amounted to about 6 Mio. €.

External funding More than 60 % of the external funding came from national research programmes. About 14 % was obtained from EU programmes and Industry, respectively.

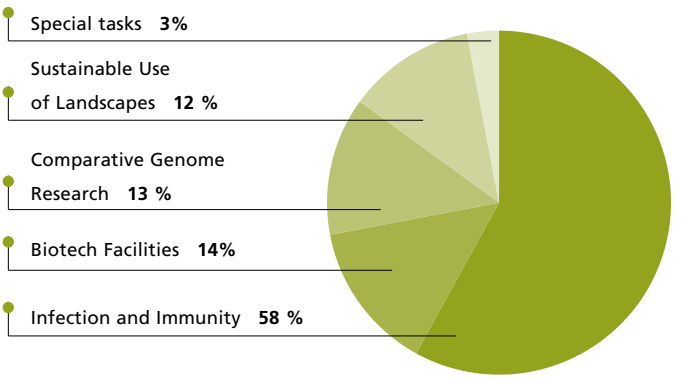
Costs per programme (in T€)

Research Area	Programme	Full Costs
Health	Infection and Immunity	27 275
	Comparative Genome Research	6 220
Total Sum		**33 495**
Earth and Environment	Earth and Environment Sustainable Use of Landscape	5 804
Total Sum		**5 804**
National and International Research Platform	Biochemical Engineering	6 932
Special Tasks		1 604
Total Sum		**47 836**

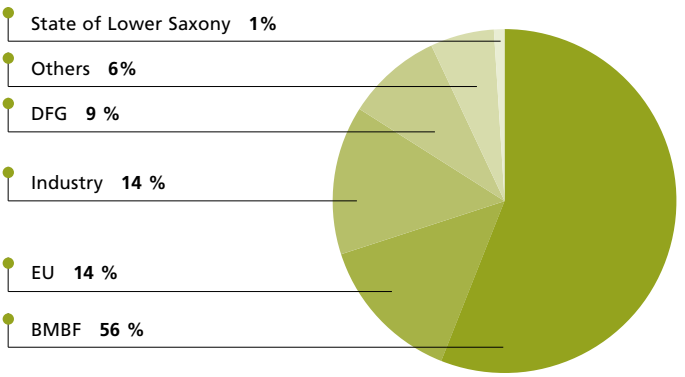
External Financing (in T€)

Source	Sum
BMBF	7 290.67
DFG	1 117.05
EU	1 851.21
Industry	1 733.07
State of Lower Saxony	109.39
Others	731.25
Total Sum	**12 832.64**

Full costs 2002



External Funding 2002 – by source



Patents / Licences In 2002, eight patents have been applied for, four each in Germany and Europe. Seven of these patents were originated in the research area "Health". During the same period seven patents have been granted, all in the research area "Health". The number of licence agreements increased two-fold, compared to the previous year.

Patents and Licences, Year 2002

	Total number	Germany	Abroad
Priority based applications (2002)	8	4	4
Priority based applications, total number	129	107	22
Granted patents (2002)	7	1	6
Total number of held patents*	74	16	58
Licence agreements (total number)	38	24	14
Licence proceeds ** (in T€)	**308**	**243**	**65**

* *This number of patents has been counted differently compared to previous reports. European patents were counted as one patent, and no longer counted for each of the countries.*

** *Including revenues from other "know-how"-transfer agreements*

Publications, Professorships, DFG-Programmes, and Guest Scientists The total number of publications remained about constant during the last years. In 2002, more articles were published in ISI-listed journals. In 2002 and 2003, several articles have been published in highly renowned journals like *Nature* or *Cell* (for more details, see under "Publications" in the section *Scientific Reports*)

Quantitative Parameters	Category	2000	2001	2002
Publications	Publications in ISI-listed journals	206	205	222
	Books and publications in other journals	47	38	27
	Total number	253	243	249
	Habilitations	2	4	3
	Dissertations	26	29	14
Calls for professorships	Calls for C3- and C4-professorships at universities	0	3	3
Special DFG-Programmes	Special fields of interest, Transregios	2	9	13
	DFG-Research Focus (SFB)	6	7	6
	Graduiertenkollegs	1	3	1
	Total number	9	19	20
Guest Scientists		116	94	107

2. Technology Transfer

The GBF has a great potential for the development of innovative products, processes and services, especially in cooperation with industrial partners. Therefore, an important goal is to foster the transfer of research results into industrial applications through technology transfer. Thus, the establishment of spin-off and start-up biotech companies, licence agreements as well as service contracts with industrial partners are important elements for the transfer of R&D results. In order to further support technology transfer activities, the GBF is a member of the BioRegioN and the "Transferkolleg Biotechnologie e.V.". Furthermore, the GBF is an active partner in BioRegioN GmbH as well as in "*BioProfil Functional Genome Analysis*".

The GBF Biotech Campus The GBF offers about 1900 sqm of laboratory and office space for spin-off and start-up companies in the 3rd and 4th floor of building Y. An important contribution to the financing of common equipment has come from the Ministry of Economics of the State of Lower Saxony. At the moment, this space is fully occupied. Therefore, the City of Braunschweig with support from the State of Lower Saxony and other organizations has built the "BioTec-Gründerzentrum" close to the GBF-Campus. Since the end of 2002, the new building is set up ready to house start-up companies.

Intellectual Property In 2002, the *Ascenion Ltd. Co.*, an IP-corporation of several Helmholtz Centres, was founded with its main office in Munich. On the GBF-Campus *Ascenion Ltd. Co*. has established a local office.

Four Helmholtz Research Centres, that are mainly active in health research, founded Ascenion. The company will merchandise the intellectual properties of these centres, but also offers its services to other institutions in the life science sector.

On 1 April, 2002, *Ascenion Ltd. Co.* opened its office with two employees on the 4th floor of the Biotec-Campus at the GBF.

Ascenion Ltd. Co. principally manages the following areas for the GBF:

- Acquisition and management of intellectual property
- Evaluation of the commercial potential of an invention before patent filing
- Development and employment of strategies for the exploitation of the GBF patent portfolio

GBF-FORUM In its 3rd year after inauguration, the GBF-FORUM continued to host an increasing number of events. During 252 days in 2002, more than 1000 events took place in this building.

List of the firms on the GBF Biotech Campus (30.09.2003)

Company	Contact person	Telephone/Fax	E-Mail Address	Homepage
Ascenion GmbH	Dr. Sabina Heim/ Tina Damm	0531-6181-961/-962; Fax: -963	she@ascenion.de tda@ascenion.de	www.ascenion.de
Hartmann Analytic GmbH	Dr. Ursula Hartmann	0531-26028-0; Fax: -28	hartmann@hartmann-analytic.de	www.hartmann-analytic.de
Cosmix GmbH	Dr. Ralf Kaufmann/ Ute Heidrich (Sekretariat)	0531-12086-0; Fax: -99	rka@cosmix.de uhe@cosmix.de	www.cosmix.de
AIMS Scientific Products GmbH	Dr. Norbert Zander	0531-2602-865; 0177-7637299; Fax: 2602-866	nza@aims-scientific-products.de	www.aims-scientific-products.de
RELIATech GmbH	Dr. Bernhard Barleon	0531-260-1832; Fax: -1833	info@reliatech.de	www.reliatech.de
Lionex GmbH	Dr. Ralf Spallek/ Dr. Eva Gebhardt-Singh	0531-6180-653/-652; Fax:2601159	msi@lionex.de	www.lionex.de
IBA Biologics GmbH	Dr. J. Bertram/Dr. Garke	0551-50672118; GBF: 170		
AMODIA Biosciences GmbH	Dr. Ulrich Krause/ Dr. Sabine Peters	0531-260-1764; Fax: -1766	sabine-peters@amodia.de ulrich.krause@amodia.de	www.amodia.com
Eugene GbR	Dr. Werner Müller	0531-6181-687	wmu@gbf.de	
BIOS- Biotechnologisches Schülerlabor	Dr. Iris Eisenbeiser/ Arntraud Meyer	0531-6181-945; Fax: -949	Bios.lab@gbf.de	

List of the firms in the "Biotec-Gründerzentrum" of the City of Braunschweig (30.09.2003)

Company	Contact person	Telephone/Fax	E-Mail Address	Homepage
Research Group Wound Healing of the TU Braunschweig	Prof. Dr. Peter Mühlradt	0531-1217-954; Fax: -958		
Vakzine Management GmbH	Dr. Albrecht Läufer	0531-2850-40; Fax: -429	jacobi@vakzine-manager.de	www.vakzine-manager.de
BioRegioN	Dr. Albrecht Läufer/ Hannes Schlender	0531-2850-415/-416; Fax: -428	braunschweig@bioregion.de	www.bioregion.de
GlycoThera GbR	Dr. Harald Conradt	0531-7996-785; 0531- 6181-287	hco@gbf.de	www.glycothera.de
Forum Functional Genome Analysis in BioRegioN	Hannes Schlender	0531-2850-416; Fax: -428	Hannes.schlender@bioregion.de	www.forum-genomanalyse.de

3. Personnel and Organization

Personnel At the end of 2002, the GBF staff comprised 636 persons with full time and part time occupation. Additionally, 101 guests worked in various projects, receiving their payment from third parties. In total, 255 scientists were working at the GBF, including 86 postdocs and 75 PhD-students.

Boards and Assemblies of the GBF The boards and assemblies of the GBF are the Board of Trustees, the Supervisory Board, the Scientific Committee and the Managing Directors.

Board of Trustees The Board of Trustees is formed by the two trustees of the GBF, the Federal Republic of Germany and the State of Lower Saxony, represented by their respective departments, the Federal Ministry of Education and Research (BMBF) and the Lower Saxony Finance Ministry.

Supervisory Board The Supervisory Board oversees the legality, expedience and economy of the management. It decides on general research goals, the principal research policy and financial affairs of the centre. It consists of a maximum of 15 members..

Scientific Committee The Scientific Committee consists of members of the Supervisory Board and external scientific experts. It advises the Supervisory Boards with regard to the R&D programme as well as general research strategy of the GBF.

Members of the Supervisory Board and the Scientific Committee (30.5.2003)

Function	Name, Title	Organisation	Locality
Chairman SB	Lange, MinDirig Dr. Peter	BMBF	Bonn
Vice-Chairman SB	Weise, MinDir Dr. Dr. Christian	NMWK	Hannover
SB	Warmuth, MinR Dr. Ekkehard	BMBF	Berlin
SB	Kuhny, RD Corinna	NMF	Hannover
SC	Apweiler, Dr. Rolf	European Bioinformatics	Cambridge
SC	Pfeffer, Prof. Dr. Klaus	University	Düsseldorf
SB + SC	Daniel, Prof. Dr. Hannelore	Technical University	München
SC	Winterfeldt, Prof. Dr. Ekkehard	University	Hannover
SB	Bilitewski, Prof. Dr. Ursula	GBF	Braunschweig
SB	Bode, Prof. Dr. Jürgen	GBF	Braunschweig
SC	Schendel, Prof. Dr. Dolores	GSF	München
SC	Birchmeier, Prof. Dr. Walter	MDC	Berlin-Buch
SC	Mann, Prof. Dr. Matthias	University	Odense/Dänemark
SB + SC	Jockusch, Prof. Dr. Brigitte	Technical University	Braunschweig
SB + SC	Schiebler, Dr. Werner	Prom. Ass. Human Genome Research	Frankfurt
SB + SC	Bitter-Suermann, Prof. Dr. Dieter	MHH	Hannover
SB + SC Vice-Chairman SB	Grummt, Prof. Dr. Ingrid	DKFZ	Heidelberg
SB + SC Chairman SC	Jäckle, Prof. Dr. Herbert	MPI	Göttingen
SC	Röllinghoff, Prof. Dr. Martin	University	Erlangen
SC	Wittinghofer, Prof. Dr. Alfred	University	Dortmund
SB + SC	Pfeiffer, Dr. Dorothea	BST	Berlin
SB + SC	Müller-Kuhrt, Dr. Lutz	AnalytiCon AG	Potsdam

Managing Directors The Managing Directors of the GBF:

- Research: Prof. Dr. Rudi Balling
- Administration: Dr. Georg Frischmann

- *Prof. Dr. Rudi Balling (le), Dr. Georg Frischmann (ri)*

Photo: Bierstedt

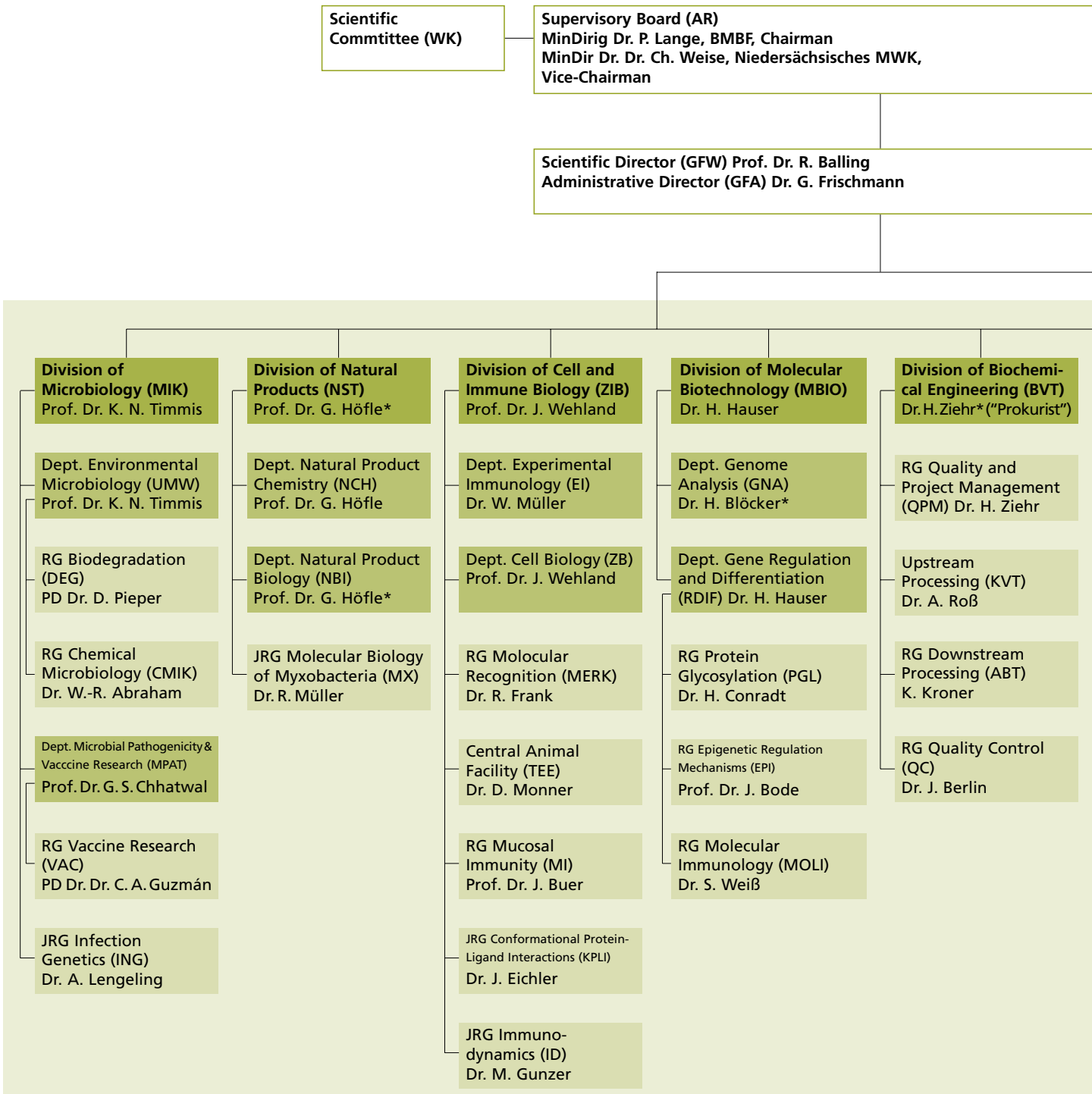
Scientists Assembly The scientists assembly of the GBF advises the Management in scientific matters. It consists of 22 elected scientists. The Managing Directors, the heads of the sections and junior research groups as well as a representative of the PhD-students are guests of the assembly. Chairman was Dr. Anton Roß (until May 2003) followed by Dr. Wolf-Rainer Abraham (since May 2003). Vice-chairman is Dr. Siegfried Weiß.

Direktorium The "Direktorium" advises the Managing Directors of the GBF in all important questions of the Centre. Members are the Managing Directors, the heads of the divisions, a representative of the junior research groups and the chairman of the Scientists Assembly.

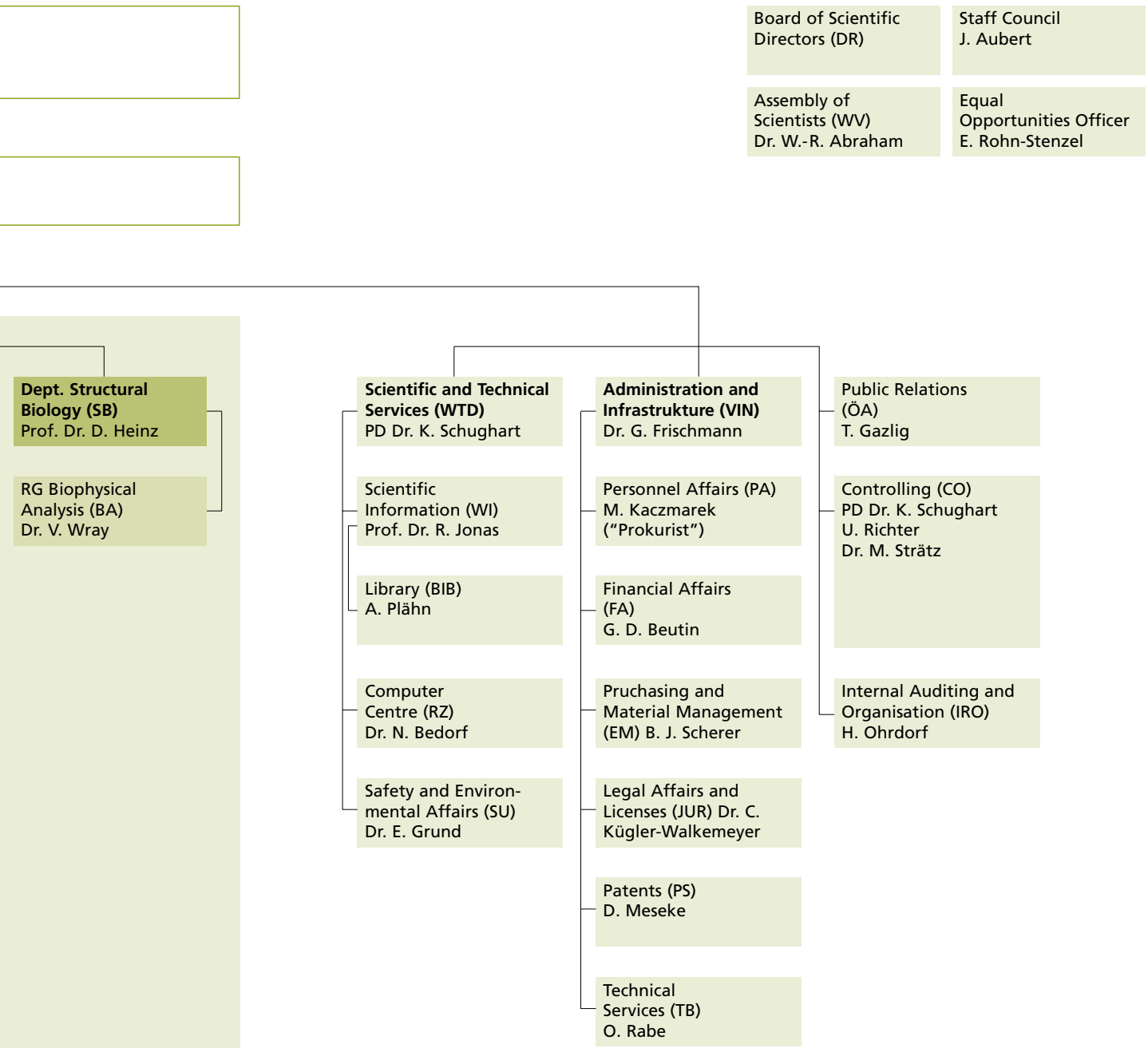
Staff Council The Staff Council has certain consultation and co-determination rights in personnel and social questions. It consists of 11 members, elected by the GBF staff. Chairman is John Aubert.

Equal Opportunities Officer is Evelyn Rohn-Stenzel.

Chart of Organisation, Status 1st Oktober 2003



SCIENTIFIC GROUPS



- *Abbreviations:*
  Dept. *Department*
  RG *Research Group*
  JRG *Junior Research Group*

- ** temporarily acting*

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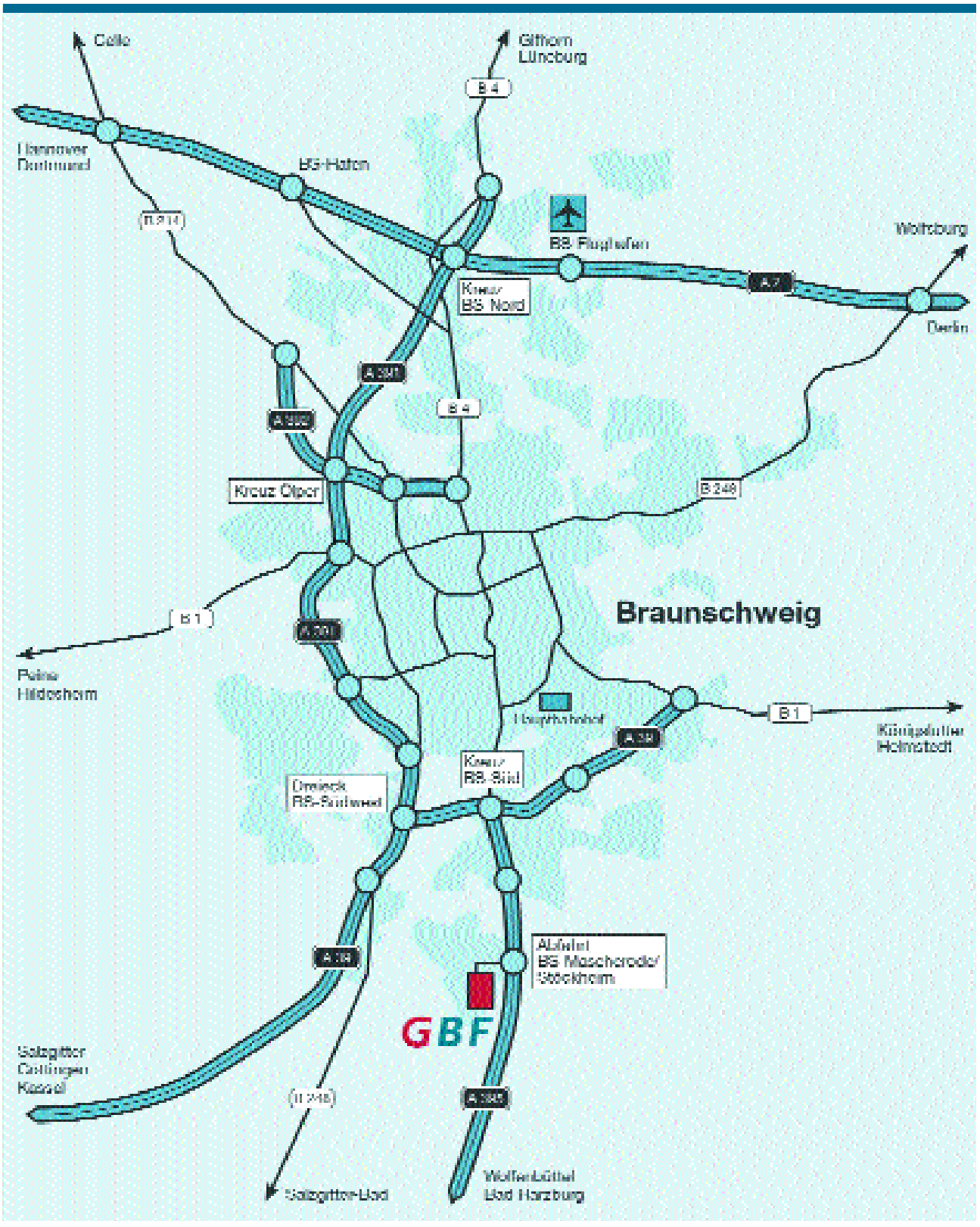

- *Site plan of the GBF*

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ANNUAL REPORT

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